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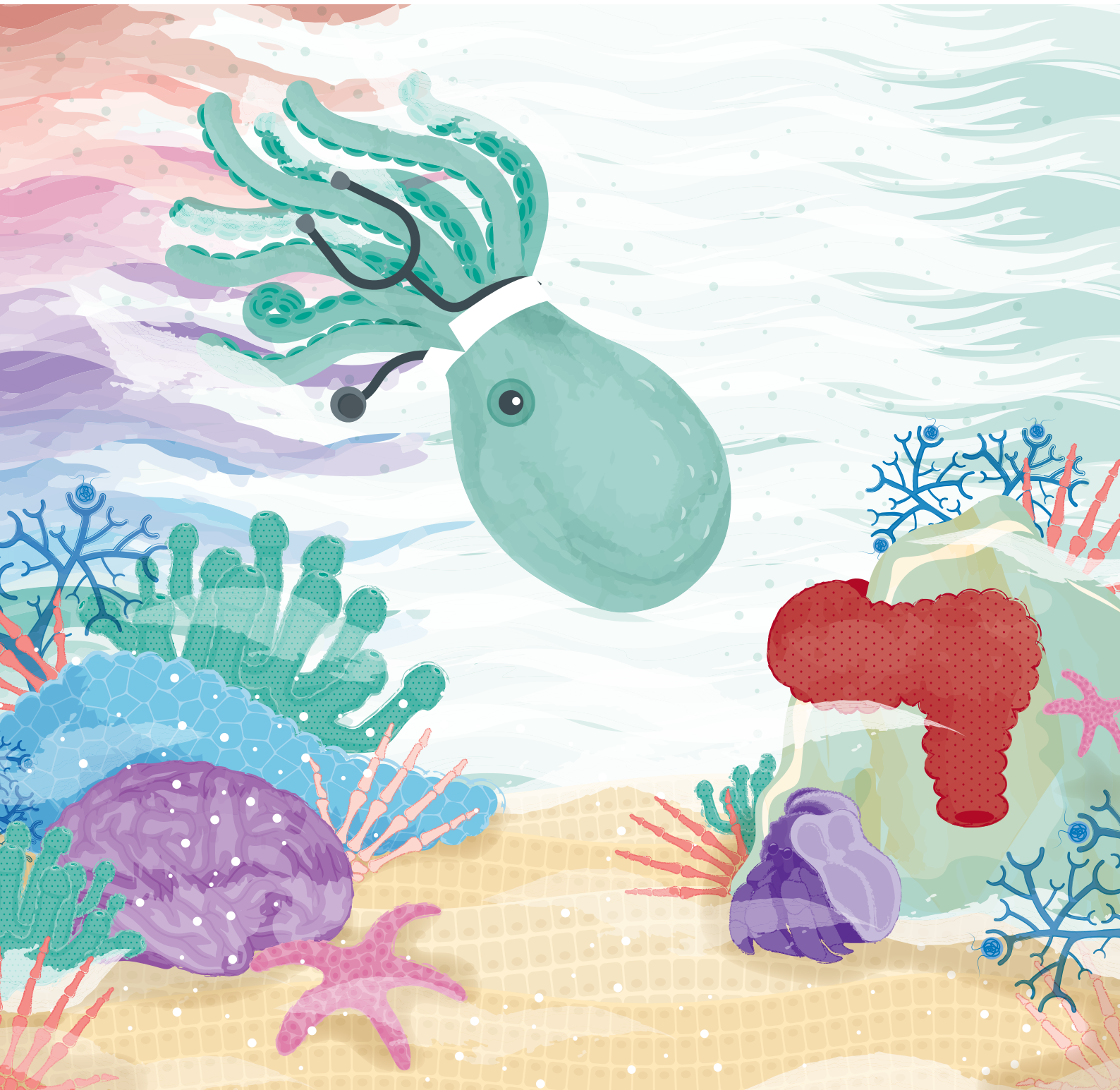

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Key Advances in Medicine

January 2018

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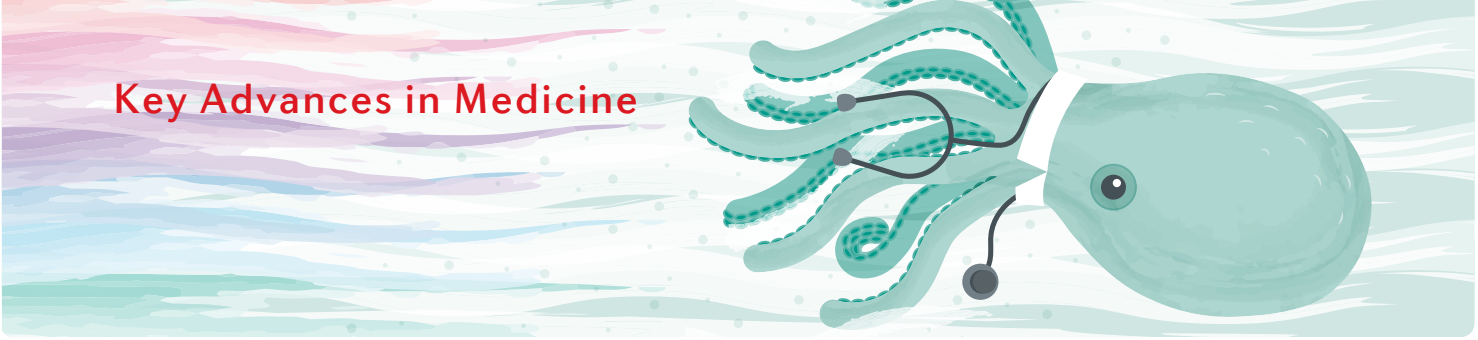

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ANTITHROMBOTIC THERAPY IN 2017

Advances in atherosclerosis, atrial fibrillation, and valvular disease

Deepak L. Bhatt

In 2017, several high-impact studies in thrombosis were published. Refinements were made in the optimal therapy for patients with stable atherosclerosis or with atrial fibrillation undergoing percutaneous coronary intervention. Risk scores to determine duration of antiplatelet therapy were developed. The potential risk of subclinical valve leaflet thrombosis was identified.

Historically, warfarin was shown to be an effective agent after myocardial infarction; however, difficulties in maintaining a therapeutic level of anticoagulation in part led to high rates of major bleeding. Antiplatelet therapy, initially with a single agent and ultimately with two agents, instead became the standard of care in patients with any form of acute coronary syndrome (ACS) for at least the first 12 months after onset of symptoms. However, with the introduction of the non-vitamin K antagonist oral anticoagulants (NOACs), interest in combination antiplatelet and anticoagulant therapy emerged (FIG. 1). Full-dose apixaban therapy in patients after ACS mostly receiving dual antiplatelet therapy (DAPT) led to unacceptable levels of bleeding with no clear benefit. However, very-low-dose rivaroxaban in a similar setting reduced ischaemic events and all-cause mortality, albeit with a significant increase in major bleeding.

This benefit in patients following ACS led to the COMPASS trial[1], which compared the previously tested very-low-dose rivaroxaban (2.5 mg twice daily) plus aspirin (100 mg once daily) versus low-dose rivaroxaban (5 mg twice daily) versus aspirin (100 mg once daily) in a broad secondary prevention population. In total, >27,000 patients with stable coronary artery disease (CAD) or stable peripheral artery disease (PAD) were studied. The independent data safety monitoring board stopped the trial earlier than planned after a mean follow-up of almost 2 years owing to a significant reduction in the primary end point of cardiovascular death, myocardial infarction, or stroke, as well as a reduction in all-cause mortality, in the rivaroxaban 2.5 mg twice daily plus aspirin group compared with the aspirin-only group[2]. No significant excess in fatal or intracranial bleeding was observed with the combination therapy, although major bleeding was significantly increased. The low-dose rivaroxaban-only treatment was not significantly more efficacious than aspirin alone. The overall efficacy and safety results with dual-pathway inhibition were consistent in the CAD[3] and PAD[4] subgroups, and the results seem to be applicable to a substantial proportion of patients with stable atherosclerosis, assuming they are at low risk of bleeding[3,4]. Of note, patients with PAD receiving the combination therapy had significant reductions in major adverse limb ischaemic events and in limb amputations — a welcome advance for these patients whose condition is challenging to manage[4].

The choice of combinations of antithrombotic therapies in patients with atrial fibrillation undergoing percutaneous coronary intervention (PCI) has been a source of great controversy. Previous studies with warfarin and rivaroxaban suggested that triple therapy with aspirin, clopidogrel, and full-dose anticoagulation with warfarin was a strategy associated with a large amount of bleeding, and that less-intense strategies seemed to provide similar efficacy but greater safety.

In the RE-DUAL PCI trial[5], >2,700 patients with atrial fibrillation undergoing PCI were randomly assigned to one of three strategies: dabigatran 110 mg twice daily plus either clopidogrel or ticagrelor; dabigatran 150 mg twice daily plus either clopidogrel or ticagrelor; or a control group receiving therapeutic warfarin, aspirin for 1–3 months, and either clopidogrel or ticagrelor. Both double-therapy strategies using dabigatran decreased the rate of major bleeding compared with the strategy of triple antithrombotic therapy with warfarin[6]. Overall efficacy seemed to be similar, although the trial, despite its size, was not well-powered for ischaemic events. Intracranial haemorrhage was also lower in the dabigatran groups than in the warfarin group. At this point, the weight of data no longer supports the use of full-dose anticoagulation plus DAPT — this strategy clearly increases the risk of important bleeding complications, which seems to overwhelm any potential reduction in ischaemic or thromboembolic events. The optimal alternative strategy is the subject of ongoing trials, but for the time being should be one of the regimens of double therapy already tested in the completed trials, such as RE-DUAL PCI.

Key advances

- Dual-pathway inhibition with aspirin plus very-low-dose rivaroxaban was shown to be more efficacious in reducing the rate of ischaemic events than aspirin alone in patients with stable coronary or peripheral artery disease[2–4]
- Double antithrombotic therapy was shown to be much safer than, and similarly efficacious to, triple antithrombotic therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention[6]
- The PRECISE-DAPT risk score is a novel method of determining the optimal duration of dual antiplatelet therapy in individual patients receiving coronary stents by trying to balance ischaemic and bleeding risks[7]
- Transcatheter aortic valves and even biological surgical valves not infrequently show subclinical leaflet thrombosis on 4D CT imaging; the clinical importance of this phenomenon is being investigated[10]

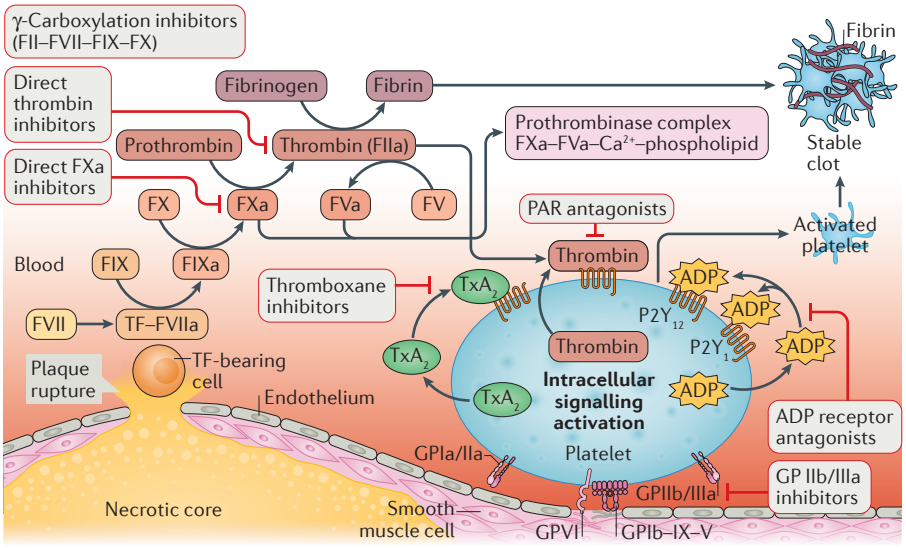

Figure 1 | **Antiplatelet and anticoagulant pathways.** Antiplatelet and anticoagulant pathways are interrelated and each contributes to thrombosis. Antithrombotic drug combinations affect these pathways, decreasing the risk of ischaemic events, but also increasing the risk of bleeding. F, factor; GP, glycoprotein; PAR, protease-activated receptor; TF, tissue factor; TxA_2, thromboxane A_2.

The optimal duration of DAPT after coronary stenting remains another source of controversy. Especially in patients with ACS, longer durations of DAPT seem to reduce ischaemic events further, although at the cost of increased rates of bleeding. After elective stenting in stable patients, newer-generation drug-eluting stents seem to be less prone to stent thrombosis than older-generation stents, creating less of a mandate for protracted DAPT on that basis. Therefore, considerable uncertainty exists about exactly how long to continue DAPT in individual patients.

The PRECISE-DAPT risk score provides an objective method to try to balance ischaemic and bleeding risks[7]. Depending on the score, a shorter or longer duration of DAPT is recommended. Quite intuitively, the score includes previous spontaneous bleeding as one of the predictors of bleeding, as well as age, creatinine clearance, haemoglobin level, and white blood cell count, which is probably a marker of illness severity. The PRECISE-DAPT score was validated in both a clinical trial and a registry. The risk calculator is freely accessible at http://www.precisedaptscore.com and provides a quantitative method of deciding on DAPT duration that some physicians might find useful.

Transcatheter aortic valve implantation (TAVI) has rapidly changed the treatment of symptomatic aortic stenosis. Subsequently, subclinical leaflet thrombosis in transcatheter aortic valves has been identified due to the advent of 4D CT[8]. Even in asymptomatic patients, this phenomenon is not infrequent; depending on the case series, subclinical leaflet thrombosis can be seen in ~10–15% of patients after TAVI if routine 4D CT imaging is performed. Autopsy data have confirmed the existence of subclinical leaflet thrombosis and that this phenomenon is indeed due to thrombus formation[9]. What remains uncertain is the clinical importance of these findings observed on imaging.

An observational study in two registries found that subclinical leaflet thrombosis occurred not only in transcatheter aortic valves but also in surgical biological valves, and was associated with an increased rate of stroke or transient ischaemic attack[10]. Anticoagulation (with either warfarin or a NOAC) seemed to be associated with resolution of this subclinical thrombus in the vast majority of patients. Ongoing randomized trials are assessing whether patients should be routinely placed on anticoagulation after TAVI, as well as prospectively evaluating whether these imaging abnormalities truly predict events such as stroke. This line of research might further improve the already excellent outcomes of TAVI. The insights gained about TAVI might also be applicable to surgical biological valves.

Thrombosis touches on many different areas of cardiovascular medicine, and antithrombotic regimens remain a central part of therapy in many cardiovascular diseases. Continued advances from clinical trials and registries refine our understanding of which patients might best be served by various antithrombotic regimens. The field of thrombosis is incredibly active and is likely to remain so for the foreseeable future.

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Competing interests statement

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CARDIAC REGENERATION IN 2017

Novel paradigms in the fight against heart failure

Francisco Fernández-Avilés

Important milestones in cardiac regenerative medicine that will define future research were reached in 2017: demonstration of adult cardiomyocyte renewal capacity, recognition of the importance of the extracellular matrix and the higher regenerative efficacy of repetitive dose protocols, and the publication of human data supporting paracrine effects of stem cell therapies and guidelines from TACTICS, the first international alliance on cardiac regenerative medicine.

Discovery of cardiac regenerative therapies is now being driven by the shift from the classical paradigm that denied the renewal capacity of mammalian adult cardiomyocytes[1]. Indeed, we now have confirmation that human heart tissue can produce new adult cells throughout the entire lifespan of the individual. Nevertheless, translating this renewal capacity into regenerative treatments is more difficult than expected. Fundamental milestones were reached in 2017 thanks to important efforts from translational science researchers and interdisciplinary, international collaboration.

> “...testing repetitive-dose protocols might be necessary before discarding a biological therapeutic option”

Shedding light on the mechanisms of cardiac regeneration, Wang *et al.* demonstrated for the first time the potential of fully mature, mammalian adult cardiomyocytes to re-enter the cell cycle and form new cardiomyocytes through a three-step process involving dedifferentiation, proliferation, and redifferentiation[2]. Under stress conditions (such as hypoxia and Ca^{2+} overload), adult cardiomyocytes expressed dedifferentiation markers (cardiac troponin I, runt-related transcription factor 1, and disabled homologue 2) and reduce their contractile capacity. This metabolic change was associated with a reduction in ATP demand and, consequently, with increased survival during ischaemia[2]. Time-lapse imaging showed the capacity of dedifferentiated adult cardiomyocytes to undergo cytokinesis[2]. Interestingly, after mitosis, new cells could produce committed progeny. Redifferentiation of new cells into contractile cells was modulated by cell-to-cell contact[2]. Specifically, the capacity of neighbouring cells to transmit Ca^{2+} transients into dedifferentiated cardiomyocytes via connexin 43 gap junctions seems to be a crucial trigger for redifferentiation[2].

The confirmation of the dedifferentiation–proliferation–redifferentiation process in adult cardiomyocytes opens the door to novel regenerative strategies. However, further research is needed to clarify the role of cellular and extracellular components of myocardial tissue in this process. Agrin, an extracellular matrix protein, was shown to have a fundamental role in cardiomyocyte growth and differentiation[3]. Agrin is a large, heparan sulfate proteoglycan involved in the development of neuromuscular junction in embryogenesis. Bassat *et al.* used various decellularized cardiac matrix extracts from postnatal mouse hearts to show that those with less agrin promoted new cardiomyocyte formation with more mature sarcomeric structures and enhanced colocalization of sarcomeric α-actinin and caveolin 3, a protein associated with t-tubules[3]. Their results in mouse cardiomyocytes and human induced pluripotent stem cell-derived cardiomyocytes suggest that treatment with agrin promotes cardiomyocyte proliferation and rejuvenation, limiting the differentiation process via modulation of dystroglycan 1, a protein that binds to the dystrophin–glycoprotein complex. In a mouse model of myocardial infarction (MI) induced by permanent ligation of the left anterior descending coronary artery, animals treated with agrin had a significant reduction of the myocardial scar area and higher left ventricular ejection fraction (LVEF) at day 35 after MI compared with untreated mice[3]. Of note, although agrin was detectable only for 3–4 days after injection, treatment peak effect was at 4–5 weeks; hence Bassat *et al.* suggest that agrin has pleiotropic effects in addition to inducing mild cardiomyocyte proliferation.

Although the discovery by Bassat *et al.* has the potential to revolutionize the way in which we can create new human myocardium in tissue engineering, the limited regenerative effect of single-dose protocols has been demonstrated for most cardiac regenerative products. Indeed, as occurs with all pharmacological treatments, the ‘one-shot’ protocol paradigm has been criticized as not sufficient to produce long-term benefits. In 2017, important steps were taken to demonstrate the feasibility and greater efficacy of repeated injections of cardiovascular regenerative products. By using a mouse model of 3-week old MI, Gou *et al.* demonstrated that repetitive doses of cardiac mesenchymal cells given at 2-week intervals resulted in higher and more consistent improvements in LVEF than a single dose, even though the single-dose group had already achieved improvements in heart function compared with vehicle-treated mice[4]. According to the analysis of collagen content of the different regions of the ventricles, the repetitive cell doses reduced the total collagen amount in noninfarcted regions, but not in the damaged myocardium, suggesting that the treatment delayed the remodelling process but did not necessarily induce tissue regeneration[4].

Key advances

- Mammalian adult cardiomyocytes have the potential to re-enter the cell cycle and form new cardiomyocytes through a three-step process of dedifferentiation, proliferation, and redifferentiation[2]
- The extracellular protein agrin modulates the ability of cardiac cells to dedifferentiate, proliferate, and mature[3]
- Repetitive administration of cardiovascular regenerative medicinal products has cumulative beneficial effects[4]
- First-generation stem cell therapies had neutral efficacy results in patients with acute myocardial infarction, with no differences between clonogenic and nonclonogenic cells, supporting the involvement of paracrine effects[5]
- The first international alliance on cardiac regenerative medicine (TACTICS) has issued recommendations to define further research from a collaborative standpoint[6]

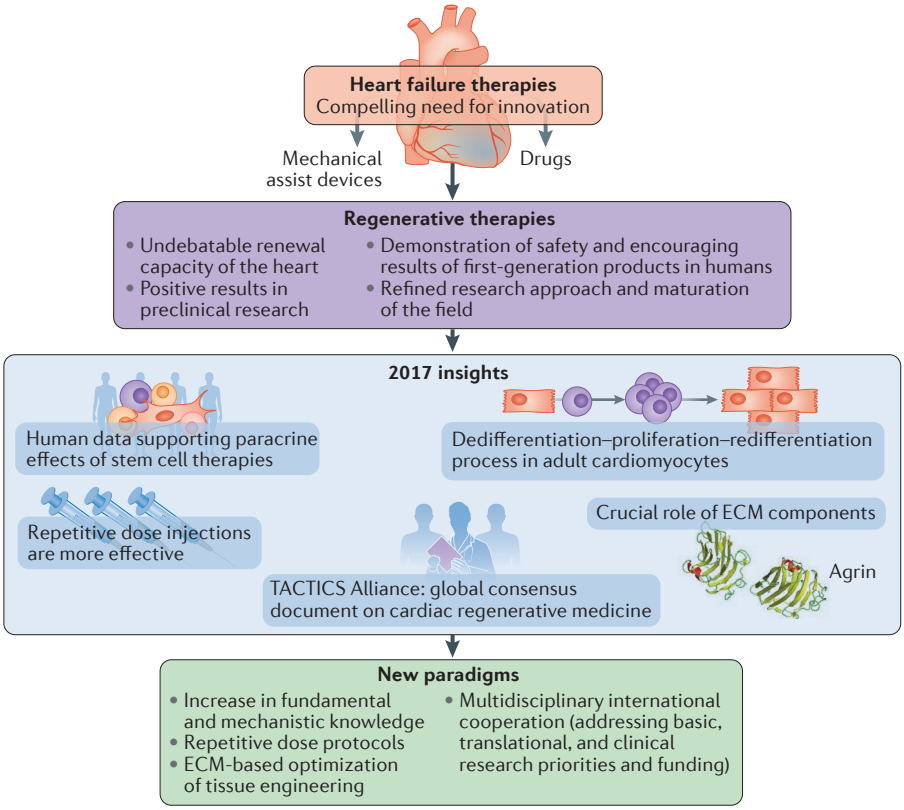

Figure 1 | **2017 insights for the evolution of cardiac regenerative medicine.** Use of regenerative therapies is supported by the demonstrated renewal capacity of the adult heart and by positive results of different products in animal models. In addition, first-generation stem cell therapeutics have been shown to be safe and modestly beneficial in humans. Important contributions published in 2017 show a more refined research approach and general maturation of the field: demonstration of the capacity of adult mammalian cardiomyocytes to dedifferentiate, proliferate, and redifferentiate[2]; identification of the important role of the extracellular matrix (ECM) protein agrin[3]; overcoming the 'one-shot' protocol paradigm[4]; clinical data supporting paracrine effects of stem cell therapies[5]; and the first global consensus document of a transnational alliance on cardiac regenerative medicine (TACTICS) with future implications for this field. These advances have challenged the main paradigms of the field medicine and have repositioned old and new biological treatments in the pipeline.

The increased efficacy of repeated cell doses indicates that testing repetitive-dose protocols might be necessary before discarding a biological therapeutic option. Ongoing clinical trials with repetitive therapeutic doses will help to translate this novel concept to the clinic.

In the clinical arena, and before awaited phase III clinical trials are published, Wollert *et al.* demonstrated in the BOOST-2 trial[5] the negligible effect of intracoronary infusion of autologous nucleated bone marrow-derived mononuclear cells (BM-MCs) on LVEF, left ventricular volume, infarct size, and regional systolic function at 6 months. No safety issues were observed in 153 patients with ST-segment elevation MI (STEMI) and left ventricular dysfunction who were randomly assigned to receive intracoronary infusion of various doses of BM-MCs 7 days after hospital admission in addition to standard therapy. Interestingly, to assess the paracrine function of the cells, the investigators also included two study groups with the same cell doses but using irradiated BM-MCs. None of these strategies improved the results obtained in the two groups receiving only standard-of-care management. Furthermore, clonogenic (that is, nonirradiated) and nonclonogenic (that is, irradiated) BM-MCs exerted the same positive effects on left ventricular parameters[5]. The trial had to be interrupted after 5 years, before reaching the sample size of 200 patients, owing to slow recruitment, emphasizing the challenge of developing stem cell therapy trials and reinforcing the evidence of the good outcomes of current standard of care in this patient population. Therefore, the results of the BOOST-2 trial do not support the use of nucleated BM-MCs in patients with STEMI, but highlight the importance of paracrine effects in cardiac stem cell-based therapy.

Finally, a definitive achievement for cardiovascular regenerative medicine was attained in 2017 with the publication of a global position paper for the comprehensive cardiovascular application of regenerative medicinal products[6]. Two years after its conception, the TACTICS Alliance issued a series of scientific statements and guidelines after a thorough critical reflection on the state of the art in cardiovascular regenerative medicine. This alliance pooled the opinion of >100 research groups and leading experts in the field, covering basic, translational, and clinical research, to analyse the available evidence and describe the priorities and challenges in cardiovascular regenerative medicine, and to provide evidence-based recommendations to guide the future application of regenerative products in the fight against cardiovascular diseases. Together with new drugs and continuous developments in mechanical cardiocirculatory support devices, cardiovascular regenerative medicine will help us to meet the compelling need for innovation in the field of heart failure management in the coming years (FIG. 1).

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The author declares no competing interests.

FURTHER INFORMATION
TACTICS Alliance: https://www.tacticsalliance.org
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

DYSLIPIDAEMIAS IN 2017

Atherogenic lipoproteins as treatment targets

Alberico L. Catapano

Research on dyslipidaemias in 2017 reaffirmed the central role of reducing the levels of atherogenic apolipoprotein B-containing lipoproteins, predominantly LDL, in preventing ischaemic cardiovascular events. However, whether increasing HDL-cholesterol levels in isolation can reduce cardiovascular risk remains to be determined.

A number of ground-breaking observations have been made in the search for treatment targets for dyslipidaemias in 2017. The questions around safety and efficacy of 'extreme' LDL-cholesterol (LDL-C) lowering, especially — but not solely — for brain function, have been fuelled by numerous epidemiological observations and *post hoc* analyses from randomized trials. However, a Mendelian randomization study reported no association between genetically determined low LDL-C levels and the risk of Alzheimer disease, dementia, or Parkinson disease[1]. Similarly, the apparent link between low levels of LDL-C and cancer, depression, or infectious disease might be explained as secondary phenomena, indicating that low LDL-C levels are a marker of the disease rather than a possible cause. Publication of the FOURIER trial[2] and a subsequent subanalysis[3] have further clarified this issue. Investigators of FOURIER[2] evaluated the effect of evolocumab or matching placebo in 27,564 patients with atherosclerotic cardiovascular disease and LDL-C levels >1.8 mmol/l on background statin therapy. Evolocumab treatment was associated with a 59% reduction in LDL-C levels and a 15% reduction in the risk of the primary end point (a composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization)[2]. Interestingly, a secondary analysis of this trial showed a monotonic relationship between achieved LDL-C levels and major cardiovascular outcomes[3]. Patients with LDL-C levels of <0.5 mmol/l had the lowest rate of cardiovascular death, myocardial infarction, or stroke compared with patients with LDL-C levels of ≥2.6 mmol/l. Importantly, no differences in adverse events were observed between all LDL-C level groups, indicating that achieving very low levels of LDL-C can further and linearly reduce cardiovascular risk without any safety concerns[3]. However, LDL-C lowering with statins has previously been shown to increase the risk of new-onset diabetes mellitus, especially for reductions of ≥30%[4]. These data are at odds with the lack of effect reported in the FOURIER study, yet on closer observation the data seem to suggest a divergence in the rate of incident diabetes between the antibody-treated group and the control group that increases with time. Further investigation is needed to address this concern.

> "...achieving very low levels of LDL-C can further and linearly reduce cardiovascular risk without any safety concerns"

A 2017 analysis of the WOSCOPS trial[5] provided further insights into the long-term cardiovascular benefits of LDL-C lowering in patients with LDL-C levels of ≥4.91 mmol/l. The original WOSCOPS study was a primary prevention trial that assessed the benefits of pravastatin in patients with hypercholesterolaemia[6]. A reduction in LDL-C levels was associated with a reduced incidence of myocardial infarction and cardiovascular death after a follow-up of 4.9 years. The new analysis exclusively included patients without a history of vascular disease, and long-term mortality outcomes for the two original treatment groups (pravastatin and placebo) were compared by stratifying patients according to baseline LDL-C levels (<4.91 mmol/l or ≥4.91 mmol/l)[5]. During the 20-year follow-up, patients allocated to pravastatin showed a significant reduction in cardiovascular outcomes and all-cause mortality compared with those allocated to placebo[5]. These reductions were greater among patients with LDL-C ≥4.91 mmol/l. Furthermore, in the subgroup of patients free of diabetes, placebo-treated patients with LDL-C ≥4.91 mmol/l showed a higher-than-predicted risk of cardiovascular events[5]. Accordingly, primary prevention among these patients should translate to a consistent long-term cardiovascular benefit, but the effect of longer times of exposure to the drug should also be taken into account.

Although statins are the preferred therapeutic strategy for lowering LDL-C levels, they have some limitations, including potential adverse events that lead to therapy discontinuation. Anti-PCSK9 monoclonal antibodies offer an alternative strategy, but are also limited by the high cost and potential for anti-drug antibody formation. Two new promising approaches for reducing LDL-C levels are now under investigation. Inclisiran is a small interfering RNA (siRNA) that works by inhibiting the translation of PCSK9. A phase II clinical study published in 2017 assessed the efficacy of inclisiran in patients with high LDL-C levels despite being on a maximal tolerated dose of statin therapy[7]. PCSK9 levels dose-dependently decreased after inclisiran administration and LDL-C levels were significantly reduced by approximately 50% from baseline[7]. Adverse events during treatment were similar between inclisiran-treated and placebo-treated patients. Larger studies of longer duration are needed to establish the long-term efficacy and safety of inclisiran.

Key advances

- Evolocumab treatment is associated with a reduction in LDL-cholesterol levels and a decrease in the rate of cardiovascular death, myocardial infarction, or stroke[2]
- The 20-year follow-up results of the WOSCOPS primary prevention trial show that long-term pravastatin therapy is associated with a significant reduction in cardiovascular outcomes and all-cause mortality[5]
- Inclisiran, a small interfering RNA that inhibits the translation of PCSK9, significantly reduced LDL-cholesterol levels in patients without increasing the rate of adverse events[7]
- The AT04A anti-PCSK9 vaccine significantly reduced total cholesterol levels, vascular inflammation, and atherosclerotic lesion size in mice[9]
- Anacetrapib, a cholesteryl ester transfer protein inhibitor, increased HDL-cholesterol levels, reduced LDL-cholesterol levels, and reduced the occurrence of a first major coronary event[10]

Another novel approach to lower LDL-C levels is active vaccination, whereby highly specific, long-lasting antibodies against PCSK9 are formed by stimulating the immune system. Unlike anti-PCSK9 monoclonal antibodies, these vaccine-mediated antibodies are not limited by potential anti-drug antibody formation. In preclinical models, a peptide-based, anti-PCSK9 active vaccination induced generation of high-affinity antibodies specific for PCSK9, reducing LDL-C levels by up to 50%[8]. In 2017, Landlinger and colleagues showed that the AT04A anti-PCSK9 vaccine reduced plasma lipid levels and inflammation, as well as atherosclerotic lesion area in mice[9]. The main limitation of this type of approach is the high variability in antibody response, necessitating the development of vaccine protocols that are able to induce a high-titre response.

Finally, the long awaited results from the large REVEAL trial[10] to assess cholesteryl ester transfer protein (CETP) inhibition were reported in 2017. Anacetrapib is the last of a series of potent CETP inhibitors being tested in an event-driven trial. Investigators of the REVEAL trial[10] evaluated the clinical efficacy and safety of anacetrapib in high-risk patients with well-controlled LDL-C levels (mean 1.58 mmol/l) treated with statins (with or without ezetimibe). During a median follow-up of 4.1 years, anacetrapib reduced the occurrence of a first major coronary event. HDL-cholesterol (HDL-C) levels significantly increased in the anacetrapib group, whereas LDL-C levels decreased by 41%, an overestimated reduction owing to the changes in LDL composition. However, an alternative, more accurate measurement showed a reduction of LDL-C levels of only 17%. Although the direct mechanism by which anacetrapib reduced coronary risk in this trial is unknown, the higher HDL-C levels achieved by anacetrapib-treated patients might not be directly responsible[10]. Indeed, the reduction in non-HDL-C observed with anacetrapib treatment would predict a 10% relative reduction in the risk of coronary death or myocardial infarction, which is similar to the reduction observed in the trial. Of note, among patients without diabetes at baseline, those treated with anacetrapib had a lower incidence of new-onset diabetes compared with placebo-treated individuals and a lower percentage of glycated haemoglobin[10]. These findings might be related to the accumulating evidence indicating that HDL-C can have a beneficial effect on glucose metabolism.

> “Larger studies of longer duration are needed to establish the long-term efficacy and safety of inclisiran”

In summary, 2017 was an exciting year of new discoveries reaffirming the central role of lowering of atherogenic lipoproteins such as LDL or large apolipoprotein B-containing lipoproteins in preventing ischaemic cardiovascular events (FIG. 1). Several studies explored new pharmalogical approaches that might pave the way to better control of LDL-C levels by improving compliance.

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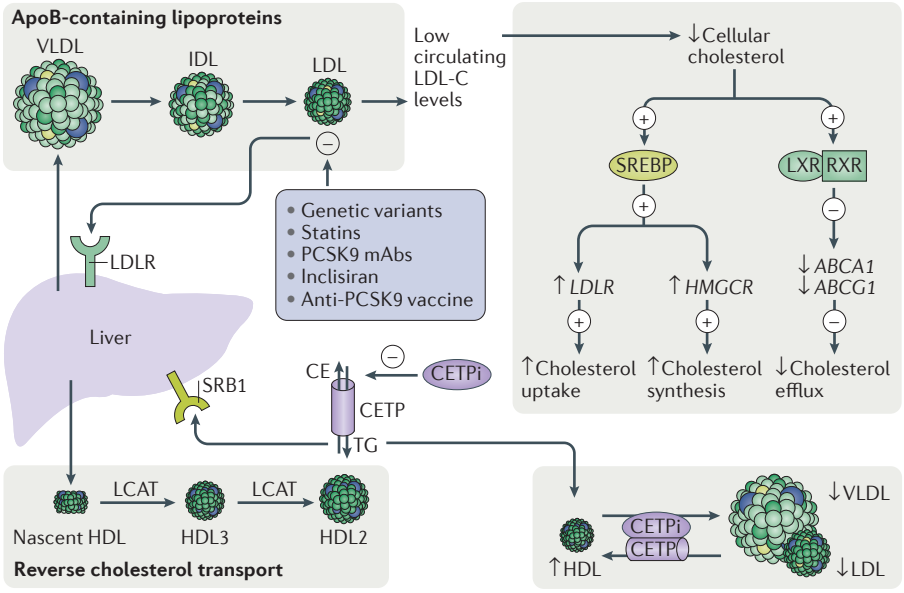

Figure 1 | **Overview of lipoprotein metabolism and effects of novel lipid-modulating approaches.** The major role of lipoproteins is to deliver lipids to peripheral tissues and return lipids for excretion or recycling. Because elevated LDL-cholesterol (LDL-C) level is a major cardiovascular risk factor, several approaches have been developed to target LDL-C levels. The most recent LDL-C-lowering strategies being investigated (including monoclonal antibodies [mAbs] and vaccines against PCSK9) allow achievement of very low LDL-C levels. Within cells, compensatory mechanisms are activated to counteract the low levels of circulating cholesterol, allowing normal cell activity. Inhibition of cholesteryl ester transfer protein (CETP) leads to an increase in HDL-cholesterol levels; however, blocking the activity of this transfer protein also results in the reduction of apolipoprotein B (apoB)-containing lipoprotein levels, which might explain the reduction of cardiovascular events observed in anacetrapib-treated patients. CE, cholesteryl ester; CETPi, cholesteryl ester transfer protein inhibitors; IDL, intermediate-density lipoprotein; LDLR, low density lipoprotein receptor; LCAT, lecithin–cholesterol acyltransferase; LXR, liver X receptor; RXR, retinoid X receptors; SRB1, scavenger receptor class B type 1; SREBP, sterol regulatory element-binding protein; TG, triglycerides; VLDL, very low density lipoprotein.

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GENETICS OF CVD IN 2017

Expanding the spectrum of CVD genetics

Heribert Schunkert

In 2017, genetic research on cardiovascular disease (CVD) produced seemingly paradoxical findings. Thanks to the continuous upscaling of genotyping and sequencing data, researchers have discovered that whereas numerous genetic variants among the general population can increase CVD risk, an individual can tolerate most severe genetic alterations.

In 2017, genetic research on cardiovascular disease (CVD) benefited — as in previous years — from the steadily increasing numbers of individuals analysed in sequencing and genome-wide association studies (GWAS)[1,2], which contributed to the emergence of three novel concepts in CVD genetics. The first is directly related to the steadily growing number of loci (or genes) identified as having genome-wide significance. Given that most genes carry variants affecting their expression levels, Pritchard and co-workers proposed that many of these variatns will eventually leave functional traces of sub-optimal expression that translate to genome-wide significance with certain traits in mega-scale GWAS[3]. In addition, Jaiswal and colleagues reported that somatic mutations can markedly increase the risk of CVD by mediating the clonal expansion of circulating leukocytes that carry a higher inflammatory burden into atherosclerotic lesions[4]. The final novel concept relates to loss-of-function (LoF) mutations; multiple partial or complete deletions of either gene are thought to exist without an obvious resulting phenotype in most individuals[5].

The publication of genotyping and phenotyping data from 500,000 individuals from the UK Biobank led to a number of new discoveries in 2017 (REFS 1,2). Together with previous efforts in the field (such as those on coronary artery disease or hypertension) the number of chromosomal loci with genome-wide significance has reached the hundreds[1,2]. The close correlation between the growing numbers of individuals studied in GWAS and the increasing number of genetic variants reaching genome-wide significance can be extrapolated to mean that all genes might carry a variant that affects a trait in one way or another, which will eventually produce a GWAS signal if the studied population is indefinitely large. Pritchard and co-workers coined the term 'omnigenic' to highlight this proposition[3]. Indeed, almost all genes might conceivably have a role in one of the diverse cell types involved in the pathogenesis of atherosclerosis (FIG. 1). Therefore, this thoughtful paper by Pritchard *et al.* reiterates that the statistical association of a genetic variant with a phenotype is merely the first step in understanding the underlying pathophysiology of the disease[3].

> "...genome-wide association studies ... contributed to the emergence of three novel concepts in CVD genetics"

Another novel concept that surfaced in 2017 was that somatic mutations might increase the risk of coronary heart disease (CHD). Jaiswal and colleagues demonstrated that the presence of clonal haematopoiesis of indeterminate potential (CHIP; defined as the expansion of a somatic blood-cell clone in an individual without other haematological abnormalities) markedly increased the risk of CVD[4]. In prospective cohorts, carriers of CHIP had a 1.9-fold increased risk of CHD. Mutations in *ASXL1*, *DNMT3A*, *JAK2*, and *TET2*, which might affect the risk of CHIP, were each individually associated with CHD. Moreover, hypercholesterolaemia-prone mice that received bone marrow from homozygous or heterozygous *Tet2* knockout mice developed larger atherosclerotic lesions in the aorta than mice that had received control bone marrow[4]. Taken together, the presence of CHIP in peripheral blood cells was associated with almost a doubling in the risk of CHD in humans and with accelerated atherosclerosis in mice[4].

A major goal of biomedicine is to understand the function of every gene in the human genome. LoF mutations disrupt a given gene, and phenotypic analysis of such knockouts can provide insight into gene function. Consanguineous unions are more likely to result in offspring carrying homozygous LoF mutations. By sequencing the protein-coding regions of 10,503 adults from Pakistan with a high consanguinity rate, Saleheen and colleagues identified carriers of homozygous LoF mutations, and performed a broad phenotypic analysis[5]. In total, they observed LoF mutations in 1,317 genes, many of which did not produce an obvious phenotype. Indeed, complete deficiency of some genes (such as *APOC3*) was associated with a favourable risk-factor profile, such as lower plasma triglyceride levels[5]. *APOC3* was also the subject of a large-scale genetics study published in 2017 (REF. 6). *APOC3* encodes apolipoprotein C-III (apoC-III), a crucial inhibitor of triglyceride lipolysis. Khetarpal and colleagues identified a missense variant in *APOC3* (A43T) that lowered triglyceride levels and protected against CHD[6]. They developed a monoclonal antibody that targets lipoprotein-bound human apoC-III, and improved circulating apoC-III clearance in mice expressing human *APOC3*. These findings reveal a novel protective mechanism by

Key advances

- The (almost) exponential rate by which genetic variants affecting complex traits are being identified resulted in the proposal of 'omnigenic' inheritance by Pritchard and colleagues[3]
- Expansion of somatic blood cell clones can markedly increase the risk of cardiovascular disease (CVD), potentially by mediating a higher inflammatory burden within atherosclerotic lesions[4]
- Almost every individual carries a loss-of-function mutation, some of which might confer protection from CVD[5–7]
- Advances in genome-editing technologies enabled correction of a mutation in the gene responsible for hypertrophic cardiomyopathy in human pre-implantation embryos[8]
- The mechanisms by which single nucleotide polymorphisms can contribute to the pathogenesis of CVD continue to be identified[9,10]

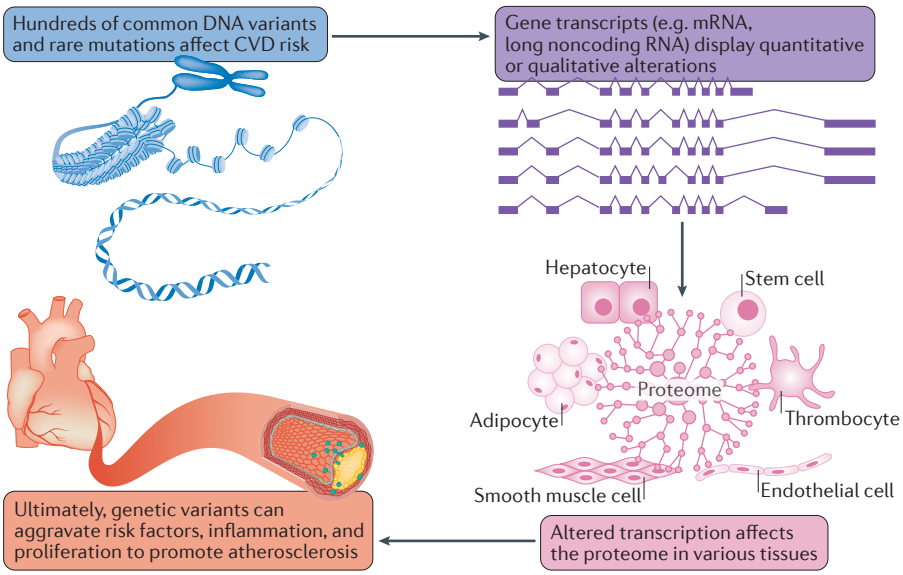

Figure 1 | **Evolution of genetic concepts underlying risk of cardiovascular disease.** The number of chromosomal loci known to affect cardiovascular disease (CVD) risk with genome-wide significance almost doubled in 2017. Likewise, the implications of genetic variations on their expression levels have become more transparent through large-scale RNA sequencing studies. Even more genetic variants will be found to affect CVD risk in the foreseeable future, given that thousands of genes have a role in one of the cell types involved in the pathogenesis of CVD, most of these genes carry variants that affect their expression level, and respective gene products interact at the protein level. These variants might include somatic mutations (those occurring in stem cells causing clonal expansion). Some of the genetic variants will have immediate mechanistic implications and, therefore, larger effects on CVD risk, whereas others might have less functional relevance. Conversely, the complete loss of a gene (knockout) can be compensated for in an individual, without an obvious phenotype. A crucial task for the future will be to unravel the mechanisms of how genetic variations propagate risk factors or lead to CVD.

which apoC-III and circulating triglyceride levels can be reduced. Likewise, LoF variants in *ANGPTL3* have been associated with reduced triglyceride, LDL-cholesterol, and HDL-cholesterol levels, a finding that was confirmed in the 2017 study by Dewey and co-workers[7]. Genetic and therapeutic antagonism of *ANGPTL3* in humans and of *Angptl3* in mice were associated with decreased levels of three major lipid fractions and decreased risk of atherosclerotic CVD.

Of course, not all mutations can be tolerated. For example, mutations in the *MYBPC3* gene can cause hypertrophic cardiomyopathy. Ma and co-workers corrected a heterozygous *MYBPC3* mutation in human pre-implantation embryos using CRISPR–Cas9 technology[8]. They induced double-strand breaks at the mutant allele and achieved successful repair by replacement with the homologous wild-type allele. The investigators concluded that more precise genome-editing techniques in conjunction with pre-implantation genetic diagnosis might increase the number of embryos available for transfer in a woman to increase likelihood of a healthy offspring. However, the gene-edited embryos obtained by Ma and colleagues were not transferred and many issues need to be resolved before refined gene-editing techniques can enter reproduction clinics[8].

> “A major goal of biomedicine is to understand the function of every gene in the human genome”

An ongoing challenge in CVD genetics is to unravel how a common genetic variant affects the risk of a late-onset disease such as myocardial infarction. To date, such mechanistic elucidation has been successful for only a few genes that primarily affect lipid levels. In 2017, several papers provided more insights into this process. Kessler and co-workers delineated how a single nucleotide polymorphism (SNP) in *GUCY1A3* affects transcription factor binding, and characterized the pathway to determine how it alters cellular function[9]. Specifically, they showed that the transcription factor ZEB1 binds preferentially to the nonrisk allele at the *GUCY1A3* locus, resulting in increased *GUCY1A3* expression at the mRNA and protein levels. The platelets of individuals homozygous for the risk variant showed impaired inhibition of aggregation by nitric oxide, thus exposing the carrier of the variant to a higher risk of myocardial infarction[9]. Similarly, Gupta and colleagues functionally characterized a variant (in the *PHACTR1* locus) that is involved in five vascular diseases: coronary artery disease, migraine, cervical artery dissection, fibromuscular dysplasia, and hypertension[10]. Through genetic fine mapping, the investigators prioritized a common SNP in the third intron of the nearby *EDN1* gene, encoding endothelin 1, as the putative causal variant, which regulates expression[10]. The known physiological effects of endothelin on the vasculature might explain the pattern of risk for the five associated diseases[10]. Together, these papers describe strategies to identify the mechanism by which a common, noncoding variant can regulate a gene and contribute to the pathogenesis of CVD.

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Competing interests statement
The author declares no competing interests.

INFLAMMATION AND CVD IN 2017

From clonal haematopoiesis to the CANTOS trial

Filip K. Swirski

In 2017, a cluster of papers have provided strong evidence in favour of the inflammation hypothesis in cardiovascular disease. From fundamental observations on clonal haematopoiesis to clinical evidence indicating that blocking an inflammatory cytokine mitigates heart disease, 2017 has been a watershed year.

When Russell Ross wrote, in 1999, that "atherosclerosis is an inflammatory disease", I think he was being intentionally provocative[1,2]. At the time, the scientific community already knew that atherosclerotic plaques contain immune cells; indeed, this observation was the cornerstone of the response-to-injury hypothesis that inflammation is the downstream consequence of events occurring at the vessel wall. However, Ross's statement was compelling in that the 'is' signified causality. Almost 20 years and thousands of studies later, the idea that inflammation causes disease has spread, because inflammatory cells and their products have been associated not only with atherosclerosis, but also with conditions that make up metabolic syndrome, including obesity, diabetes mellitus, and hypertension. This year, a cluster of independent but complementary studies have provided some of the most compelling evidence to date in support of Ross's now-famous proclamation.

With a few exceptions, all immune cells originate from haematopoietic stem cells and progenitor cells in the bone marrow. Lymphocytes such as T and B cells mature in the thymus and spleen, respectively, but myeloid cells — comprising monocytes and neutrophils — leave the bone marrow fully formed. In the circulation, myeloid cells can cross the vascular endothelium at sites of disease predilection, directly contributing to lesion growth. Although preclinical and human studies have consistently linked leukocytes to risk of cardiovascular disease (CVD), two papers published this year have added another dimension to our evolving understanding of how leukocytes might promote disease[3,4]. These two papers build on a study from 2014, which showed that elderly individuals accumulate mutated haematopoietic clones in the blood that are associated with an increased risk of haematological cancer and all-cause death potentially related to the cardiovascular system[5]. The mutations occur in several genes, but among the most frequently mutated is *TET2*, which codes for the epigenetic regulatory enzyme methylcytosine dioxygenase TET2, which has a crucial role in DNA demethylation, possibly inhibiting haematopoietic stem cell renewal. The question, then, is whether clonal haematopoiesis contributes to CVD and, if so, how?

The first paper, published early this year, focuses on the mechanism of leukocyte contribution to atherosclerosis[3]. Using mouse models, the investigators found that haematopoietic cells lacking TET2 outcompete wild-type cells and accelerate atherosclerosis. In other words, TET2 deficiency gives haematopoietic stem cells an advantage over their wild-type counterparts, which might be expected given the function of TET2 in inhibiting cell renewal. To dig deeper, the investigators asked how the haematopoietic clones aggravate atherosclerosis, and found that TET2-deficient macrophages are more inflammatory than wild-type macrophages because TET2-deficient macrophages produce more IL-1β, which leads to increased monocyte recruitment and larger lesions[3]. The study does not address the curious observation that the competitive advantage conferred by TET2 deficiency did not produce higher overall leukocyte numbers in the blood compared with a wild-type mouse, but does provide a compelling argument for how the absence of TET2 in haematopoietic cells aggravates atherosclerosis. The second paper, published by the same team behind the initial 2014 study on clonal haematopoiesis, is both clinically and mechanistically focused[4]. The investigators performed whole-exome sequencing on blood samples from hundreds of patients for whom data on clinical outcomes were available, and concluded that the presence of clonal haematopoiesis is associated with almost a doubling in the risk of coronary heart disease. The investigators further show that mice lacking TET2 in haematopoietic cells develop larger atherosclerotic lesions than mice with wild-type haematopoietic cells, possibly because of increased leukocyte recruitment to atherosclerotic lesions[4]. The data in both papers suggest that, as we age, stem cells carrying particular mutations slowly outcompete other stem cells and thus generate a progressively larger share of the leukocyte pool. In the specific example of *TET2* mutations, this process produces a progeny that is more inflammatory and atherogenic than normal leukocytes. It is possible that other mutations shape cell function in different, maybe even atheroprotective, ways.

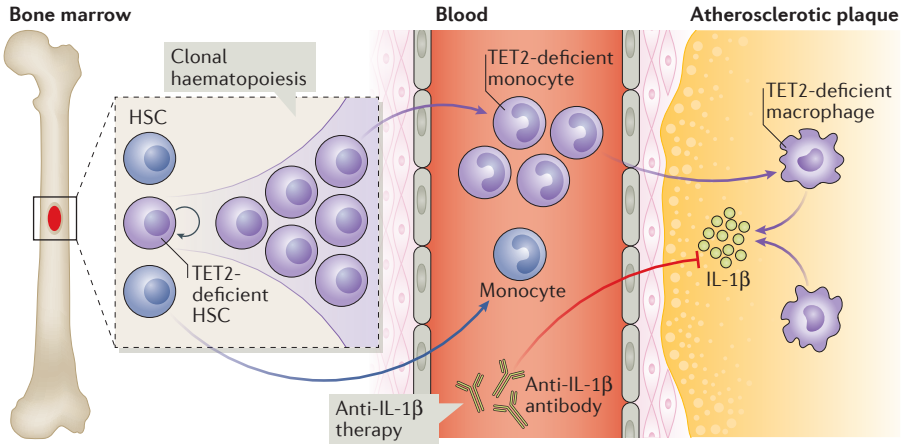

Figure 1 | **Inflammation in cardiovascular disease.** Summary of the major insights gained in 2017 on the role of inflammation in cardiovascular disease. The bone marrow is a major site of leukocyte production. Two studies have shown that some haematopoietic clones expand through a process called clonal haematopoiesis[3,4]. This process gives rise to monocytes that can infiltrate atherosclerotic lesions, where they differentiate to IL-1β-producing macrophages. In 2017, the CANTOS trial[6,7] showed that blocking the inflammatory cytokine IL-1β lowers the incidence of cardiovascular events. HSC, haematopoietic stem cell; TET2, methylcytosine dioxygenase 2.

Key advances

- Methylcytosine dioxygenase 2 (TET2) deficiency leads to clonal haematopoiesis that accelerates atherosclerosis in mice[3]
- In humans, age-associated clonal haematopoiesis predicts cardiovascular events; mice with TET2 deficiency develop accelerated atherosclerosis[4]
- Blocking the inflammatory cytokine IL-1β mitigates cardiovascular disease in patients with a history of myocardial infarction[6]
- Patients whose levels of C-reactive protein in plasma decline in response to IL-1β-blocking treatment have a more dramatic reduction in the incidence of cardiovascular events[7]

An enduring concept in immunology emphasizes functional balance and diversity. It seems self-evident that some cells produce cytokines that fuel inflammation, whereas others produce cytokines that dampen it. T-cell and macrophage subsets are often defined by their relatively opposing or complementary roles; the optimal response to infection or injury, the thinking goes, requires finely calibrated heterogeneous activities. Although clonal haematopoiesis brings to mind the rogue proliferation that occurs in cancer, clonal haematopoiesis also raises questions closely related to the concept of functional diversity. Does a diverse repertoire of haematopoietic clones preserve a healthy balance of immune processes, and, conversely, does depletion or even collapse of diversity (presumably because specific clones preferentially survive and thrive) jeopardize that balance? Moreover, if clonal haematopoiesis is a homogenizing and destabilizing process, is it also pervasive? Does its influence disseminate beyond leukocytes and immunity? Systems in the body are, at least under homeostatic conditions, irrevocably intertwined and connected. The nature of those connections, and how they can be altered, curtailed, exploited, or circumvented in a less-diverse haematopoietic environment, deserves attention. And what drives clonal haematopoiesis in the first place? Ageing, yes. But ageing without context is only the passage of time. What accompanies ageing that evokes this progressive clonality?

Although these questions will take years to answer, one thing has been settled in 2017: anti-inflammatory therapy reduces the incidence of cardiovascular events. In the CANTOS trial[6,7], patients with a history of myocardial infarction and high C-reactive protein (CRP) levels in plasma who received canakinumab — an antibody against the inflammatory cytokine IL-1β — had a significantly lower incidence of recurrent cardiovascular events compared with patients receiving placebo. The overall benefit was modest, with no change in all-cause mortality, in part because patients receiving the antibody were more likely to die from infection[6]. However, when patients were stratified according to the magnitude of the changes in CRP level after treatment, the reductions in cardiovascular mortality and all-cause mortality with canakinumab treatment were more dramatic in those patients with the most robust reductions in CRP level[7]. The clinical success of the CANTOS trial, although modest, is underscored by its conceptual triumph. We finally have a definitive demonstration that inflammation drives CVD in humans. One wonders what else is possible. IL-1β is only one cytokine in a constellation of functionally diverse ligands and receptors. Would the advantages of blocking IL-1β differ in patients stratified according to their haematopoietic clonal composition, with people harbouring the most aggressive TET2-deficient and IL-1β-producing haematopoietic clones reaping the most benefit? Or should therapy target clusters of cytokines operating upstream of IL-1β? To the extent that CVD arises as a result of disequilibrium within the immune system, restoring balance and diversity might require the modulation of multiple factors. Successful therapy might necessitate evaluating an individual's immune composition with high precision on the one hand, and delivering a cassette of immune-altering reagents on the other.

2017 has been a watershed year for the inflammation hypothesis in CVD, from the identification of how haematopoietic cells might aggravate disease to clinically targeting a major cytokine produced by those cells (FIG. 1). 2017 has been also a year that marked groundbreaking discoveries for our understanding of how the haematopoietic system coalesces with atherosclerosis and its complications. Many questions remain, but now, more than ever, there is a clear roadmap ahead.

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The author declares no competing interests.

REVASCULARIZATION IN 2017

Technical and diagnostic improvements in PCI: more pieces in the puzzle

Antonio Colombo and Antonio Mangieri

The past year provided strong evidence on the use of the instantaneous wave-free ratio to determine the severity of coronary artery disease, the improving outcomes of contemporary percutaneous coronary intervention, the increased risk of thrombosis with bioresorbable vascular scaffolds, and the benefits of a simple revascularization strategy in cardiogenic shock.

The instantaneous wave-free ratio (iFR) is an easier and more patient-friendly technology than the fractional flow reserve (FFR) and also overcomes some of the limitations associated with FFR. This diagnostic tool estimates the resistance caused by a stenosis and the total resistance of the coronary vessel measured during a wave-free period

> The AIDA trial in 2017 reported an increased risk of scaffold thrombosis using the Absorb BVS...

— that is, a part of the cardiac cycle during which the competing forces (waves) that affect coronary flow are quiescent, meaning that pressure and flow are linearly related. In the presence of a flow-limiting stenosis, the ratio of blood pressure distal and proximal to the stenosis diverges over the wave-free period; a normal ratio is 1.0, and an iFR value <0.90 suggests flow limitation. In 2017, iFR and FFR were compared in the DEFINE-FLAIR[1] and iFR-SWEDEHEART[2] trials, which had similar designs. An iFR-guided revascularization strategy was noninferior to an FFR-guided procedure with respect to the rate of major adverse cardiac outcomes at 1 year of follow-up in both studies[1,2]. A higher number of stenoses were evaluated in the iFR group because of less adenosine-related chest discomfort compared with the FFR group. The use of iFR resulted in shorter procedural times and was associated with a lower rate of procedural signs and symptoms compared with the use of FFR[1,2].

The optimal performance of modern drug-eluting stents was demonstrated in the 2017 SYNTAX II trial[3], a single-arm, multicentre, all-comers, open-label registry that included patients with *de novo* three-vessel disease treated with the best percutaneous approach available, comprising ischaemia-driven chronic total occlusion recanalization, FFR-guided and/or iFR-guided procedures, use of intravascular ultrasonography, and implantation of Synergy stents (Boston Scientific; a drug-eluting stent with an abluminal bioabsorbable polymer coating). The outcome of this cohort was compared with the historical percutaneous arm of the SYNTAX I trial[4], in which the Taxus Express stent (Boston Scientific) was used. The SYNTAX II strategy was superior to the equipoise-derived SYNTAX I percutaneous coronary intervention (PCI) cohort at 1 year of follow-up[3]. The difference was driven by significant reductions in the rates of periprocedural myocardial infarction (0.2% versus 3.8%; $P<0.001$), need for any revascularization within 1 year of follow-up (13.7% versus 8.2%; $P=0.015$), and definitive stent thrombosis (0.7% versus 2.6%; $P=0.045$). In an exploratory analysis at 1 year, PCI with the SYNTAX II strategy was associated with clinical outcomes similar to those of the equipoise-derived SYNTAX I CABG surgery cohort at 1 year of follow-up[3].

After the initial positive results for the treatment of coronary lesions using bioresorbable vascular scaffolds (BVS)[5], the AIDA trial[6] in 2017 reported an increased risk of scaffold thrombosis using the Absorb BVS (Abbott Vascular) compared with a conventional everolimus-eluting stent (3.5% versus 0.9%; $P<0.001$) in a contemporary cohort of patients. This trial had deep practical implications because the Absorb BVS was withdrawn from the market shortly after the publication of this study. The causes of the increased rate of thrombosis with the Absorb BVS are only partially understood, but some concerns have been raised about the optimal preparation of the lesion and insufficient postdilatation, because the residual diameter stenosis was ≥30% in patients with BVS thrombosis[6]. In addition, the resorption process might create a prothrombotic milieu. A longer duration of dual antiplatelet therapy might be required, given that 79% of patients with BVS thrombosis were not receiving dual antiplatelet therapy at the time of a late event. Taking into account the lack of advantage with respect to clinical safety, difficulties in the scaffold deliverability, longer procedural times, and cumbersome devices needed to assure optimal BVS implantation, little justification exists to prefer these devices over modern and safe drug-eluting metallic stents. Nevertheless, despite these negative results, we should not dismiss the new-generation BVS with thinner struts and improved tolerance to overdilatation, which are being evaluated in pilot registries.

The CULPRIT-SHOCK trial[7] in patients with acute myocardial infarction and cardiogenic shock demonstrated that percutaneous treatment of the culprit lesion alone is associated with a lower 30-day risk of a composite of death or severe renal failure leading to renal-replacement therapy compared with

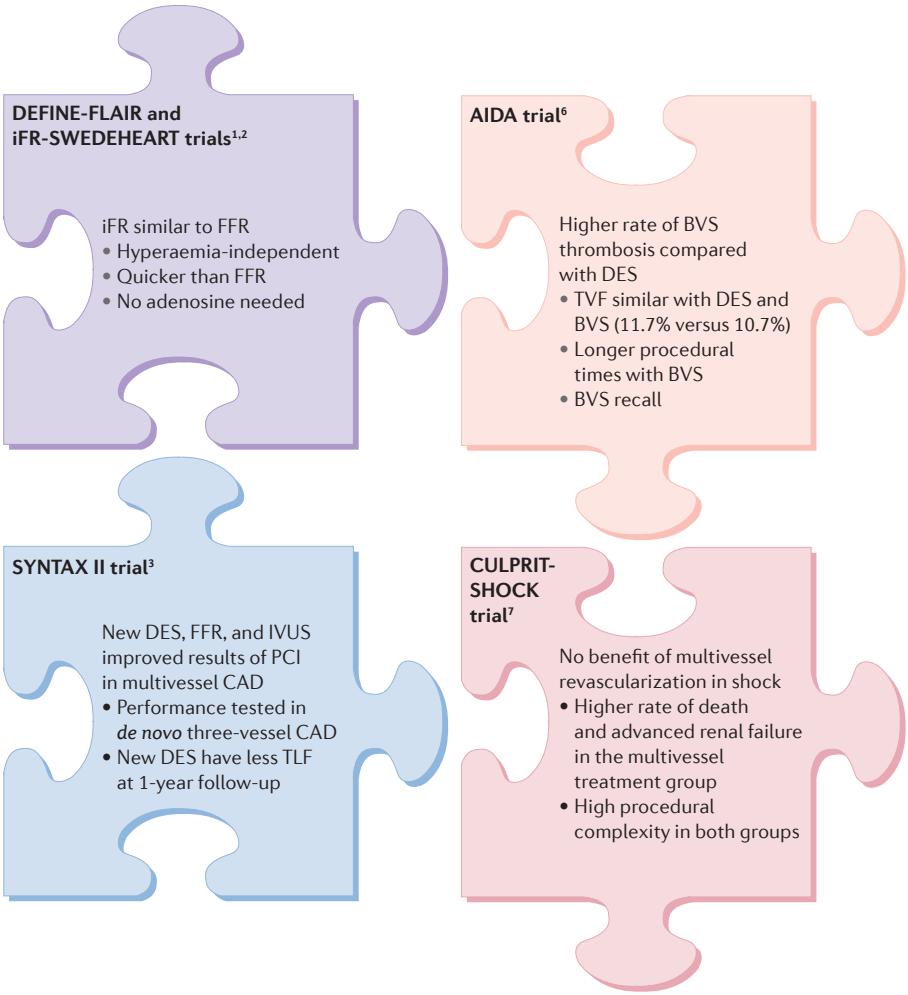

Figure 1 | **New pieces added to the puzzle of coronary revascularization.** The top trials from 2017 in the field of percutaneous revascularization and their main results. BVS, bioresorbable vascular scaffold; CAD, coronary artery disease; DES, drug-eluting stent; FFR, fractional flow reserve; iFR, instantaneous wave-free ratio; IVUS, intravascular ultrasonography; PCI, percutaneous coronary intervention; TLF, target-lesion failure; TVF, target-vessel failure.

Key advances

- In the DEFINE-FLAIR and iFR-SWEDEHEART trials, percutaneous coronary intervention (PCI) guided by the instantaneous wave-free ratio was noninferior to PCI guided by fractional flow reserve (FFR)[1,2]
- The SYNTAX II trial confirmed that use of intravascular ultrasonography, FFR, and new drug-eluting stents improves the outcome of patients treated with PCI[3]
- The AIDA trial showed a higher rate of thrombosis with bioresorbable vascular scaffolds than with drug-eluting stents[6]
- The CULPRIT-SHOCK trial demonstrated that, in patients with cardiogenic shock and multivessel disease, revascularization of the culprit lesion alone is associated with a better short-term outcome than multivessel revascularization[7]

immediate multivessel PCI (45.9% versus 55.4%; $P=0.01$), which has important clinical implications. Of note, a chronic total occlusion was present in 24% of patients in the multivessel group, and complete revascularization was successful in 81% of the patients. The high rate of these complex revascularization procedures in unstable patients with no mechanical support (72.2% of unstable patients underwent the procedure with no mechanical support) might have affected the final results. Although complete revascularization seems to offer a benefit in stable patients[8], the higher amount of contrast, the longer procedural time, and the greater risk of slow flow in multiple myocardial territories in critical patients can have detrimental effects. Guidelines on interventions in patients with cardiogenic shock will need to take these important findings into consideration.

In summary (FIG. 1), 2017 has provided new evidence about the use of iFR as a novel diagnostic tool in the armamentarium of interventional cardiologists[1,2]. In patients with cardiogenic shock and multivessel coronary disease, revascularization of the culprit lesion alone has been demonstrated to be safer than a multivessel revascularization strategy[7]. The high rate of thrombosis with BVS in the AIDA trial[6] should act as a catalyst to improve BVS technology. Looking ahead, 2018 will provide new evidence about indications for revascularization in stable coronary artery disease with the publication of the ISCHEMIA trial[9]. Other ongoing trials will provide further data about different durations of dual antiplatelet therapy after implantation of drug-eluting stents.

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ACUTE LYMPHOBLASTIC LEUKAEMIA IN 2017

Immunotherapy for ALL takes the world by storm

David T. Teachey and Stephen P. Hunger

In 2017, three groundbreaking immunotherapies for relapsed and/or refractory B-cell acute lymphoblastic leukaemia (ALL) were approved based on impressive outcomes observed in clinical trials. Additional breakthroughs included seminal research into ALL genomics and the importance of adherence to chemotherapy, which will have direct implications for clinical care.

2017 witnessed an unprecedented increase in the number of treatments available for patients with acute lymphoblastic leukaemia (ALL), with three novel therapies receiving full FDA and/or EMA approval: inotuzumab ozogamicin, blinatumomab, and tisagenlecleucel (TABLE 1). For comparison, only three agents had received FDA approval for the treatment of *BCR–ABL1*-negative ALL in the previous 25 years. Whereas immune-checkpoint inhibitors are arguably the greatest breakthrough in the treatment of solid tumours, targeted immunotherapies directed at surface antigens of leukaemic blasts have remarkable efficacy in patients with relapsed and/or refractory B-cell ALL (R/R B-ALL).

Inotuzumab ozogamicin, an anti-CD22 antibody conjugated to a calicheamicin-class cytotoxic drug, was initially shown to be active against R/R B-ALL in 2016, with the reporting of results from the phase III INO-VATE trial in 326 adult patients[1]. An intention-to-treat analysis in the first 218 patients demonstrated a markedly higher complete remission (CR) and CR with incomplete haematological recovery (CRi) rate with inotuzumab ozogamicin versus standard intensive chemotherapy (CR+CRi rate: 80.7% versus 29.4%, $P<0.001$; CR rate: 35.8% versus 17.4%; TABLE 1)[1]. Among the entire study cohort, inotuzumab ozogamicin also improved the median progression-free survival (5.0 months versus 1.8 months; $P<0.001$) and overall survival durations (7.7 months versus 6.7 months; $P=0.04$)[1]. As with other antibodies conjugated to calicheamicin, a high rate of hepatotoxicity, specifically veno-occlusive disease (VOD; sinusoidal obstruction syndrome), was a concern with inotuzumab ozogamicin[1]. In 2017, an in-depth analysis of the INO-VATE data revealed that hepatotoxicity (all grades) was frequent with both inotuzumab ozogamicin and standard chemotherapy, affecting 51% and 34% of patients, respectively[2]. The rate of VOD, however, was markedly higher with inotuzumab ozogamicin (13% versus <1%), with 82% of events being of grade ≥3 (REF. 2). VOD mostly occurred in patients who had undergone haematopoietic stem-cell transplantation (HSCT) before or after receiving inotuzumab ozogamicin; additional risk factors included conditioning with two alkylating agents and pre-HSCT serum bilirubin levels equal to or greater than the upper limit of normal[2]. This improved understanding of the toxicity profile of inotuzumab ozogamicin will guide clinical practice and the design of future trials. Indeed, the multiple newly approved therapies for R/R B-ALL provide physicians with alternatives to inotuzumab ozogamicin when treating patients with a high baseline risk of VOD. Additionally, in ongoing trials, researchers are investigating the ability of defibrotide to prevent VOD in patients at high risk, including those treated with inotuzumab ozogamicin before HSCT (NCT02851407).

Blinatumomab is a bi-specific T-cell-engager (BiTE) antibody construct that can link $CD19^+$ B cells with $CD3^+$ T cells, leading to a cytotoxic T-cell response against malignant $CD19^+$ blasts. 2017 saw the publication of results from TOWER[3], a phase III trial in which 405 adults with R/R B-ALL were randomly assigned (2:1) to receive blinatumomab or standard-of-care chemotherapy. Similarly to inotuzumab ozogamicin, blinatumomab improved the CR rate (34% versus 16%; $P<0.001$) and median overall survival (7.7 months versus 4.0 months; $P=0.01$; TABLE 1)[3]; although, direct comparisons of the INO-VATE and TOWER outcomes are not possible as the trials had different eligibility criteria. Blinatumomab also received regular FDA approval (after accelerated approval in 2014) for the treatment of children and adolescents with R/R B-ALL based on phase I/II trial data published in late 2016 (REF. 4), and approval was extended to *BCR–ABL1*-positive ALL based on results from the ALCANTARA trial[5].

Various chimeric antigen receptor (CAR)-T-cell products directed at CD19 have demonstrated remarkable efficacy in patients with R/R B-ALL. In 2017, the autologous CAR-T-cell therapy tisagenlecleucel became the first genetically engineered adoptive cell therapy to be approved by the FDA, on the basis of a CR+CRi rate of 83% among 63 children or young adults with R/R B-ALL in the single-arm ELIANA trial[6] (TABLE 1). Overall survival was 89% at 6 months and 79% at 12 months[6]. Notably, blinatumomab

Table 1 | **Immunotherapies approved in 2017 for the treatment of B-ALL**

Agent	Trial	Approved indication	Outcome for approval
Inotuzumab ozogamicin (anti-CD22 antibody–drug conjugate)	INO-VATE[1]	Adults with R/R B-ALL*	CR rate: 35.8% (89.7% MRD–) vs 17.4% (31.6% MRD–) with chemotherapy
Blinatumomab (anti-CD19/CD3 bi-specific T-cell-engager)	TOWER[3]	Children and adults with R/R B-ALL‡	Median overall survival: 7.7 months vs 4.0 months with chemotherapy
Tisagenlecleucel (anti-CD19 chimeric antigen receptor T cells)	ELIANA[6]	Patients aged ≤25 years with R/R B-ALL (after ≥2 prior lines of therapy)§	Overall remission rate: 82.5%; 63% CR,19% CRi (100% MRD–)

CR, complete remission; CRi; CR with incomplete haematological recovery; MRD–, minimal residual disease negative; R/R B-ALL, relapsed and/or refractory B-cell acute lymphoblastic leukaemia. *Inotuzumab ozogamicin received EMA approval in 2017. ‡Blinatumomab received accelerated approval by the FDA in 2015 and full approval in 2017, and was approved by the EMA in 2015. §Tisagenlecleucel is currently approved by the FDA only.

Key advances

- In 2017, three novel and distinct immunotherapies, inotuzumab ozogamicin, blinatumomab, and tisagenlecleucel, were approved for the treatment of relapsed and/or refractory B-cell acute lymphoblastic leukaemia (ALL) based on compelling results from international clinical trials[1,3,6]
- Comprehensive genomic profiling of a large cohort of patients with T-cell ALL enabled the identification of novel driver genes and dysregulated pathways that are potential therapeutic targets[9]
- A study challenging common practice revealed that clinical dogma can sometimes have important, easily avoidable consequences for treatment adherence and patient outcomes[10]

and CAR T cells share two common and potentially life-threatening toxicities that are not seen with chemotherapy or inotuzumab ozogamicin: neurotoxicity and cytokine-release syndrome (CRS). In the ELIANA trial[6], transient but severe neurotoxicity (grade ≥3), including seizures and encephalopathy, occurred in 15% of patients; no grade 5 neurotoxicity occurred. 49% of patients developed grade 3–4 CRS, which was successfully managed with the anti-IL-6-receptor antibody tocilizumab[6]. On the basis of evidence from multiple other trials[7], tocilizumab was approved simultaneously with tisagenlecleucel for the treatment of CAR-T-cell-associated CRS. Importantly, the use of tocilizumab can avert the need for immunosuppressive corticosteroids, which potentially reduce the efficacy of T-cell-based therapies (such as blinatumomab and tisagenlecleucel).

Although the outcomes with tisagenlecleucel are compelling, some patients relapse with $CD19^-$ blasts. Moreover, others are unable to receive the product owing to comorbidities or impaired T-cell function after prior cytotoxic therapy, which precludes the manufacture of a usable product. Such manufacturing issues could potentially be avoided by use of universal CAR T cells. In 2017, the first results relating to the use of such 'off-the-shelf' CAR T cells in humans were published. Qasim *et al.*[8] transduced allogeneic, non-HLA-matched donor cells with a lentiviral anti-CD19 CAR construct, and performed TALEN-mediated gene editing of the T-cell receptor α-chain and CD52 loci to reduce the risk of graft-versus-host disease (GVHD); two infants with R/R B-ALL who were treated with the resulting T cells achieved remission and were successfully bridged to HSCT, although both developed mild GVHD and one had prolonged B-cell aplasia. Owing to these toxicities and the theoretical risk of genotoxicity from the TALEN-induced translocations, the investigators recommended that this product be used only as a bridge to transplantation, and not as a stand-alone therapy[8].

T-cell ALL (T-ALL) accounts for 15–25% of ALL cases. Effective immunotherapy has been more challenging to develop for T-ALL, owing to the severe risk of immunocompromise with elimination of nonmalignant T cells and the potential for fratricide between T-cell clones. In 2017, Liu *et al.*[9] reported the first large-scale integrated genomic analysis of paediatric T-ALL. Whole-exome sequencing, copy-number analysis, and RNA sequencing of baseline and post-remission specimens were performed in 264 children and young adults with *de novo* T-ALL; 106 putative driver genes were identified, 50% of which had not been previously associated with childhood T-ALL[9]. The spectrum of genetic alterations was heterogeneous between patients, although cancers could be categorized into ten different groups according to the functional activation of potentially targetable signalling pathways[9]. Moreover, strong correlations between different T-ALL subgroups, the development stage of T-ALL blasts, and particular genetic alterations were discovered[9]. This comprehensive analysis provides multiple potentially translatable findings.

New therapies are not the only means to improve patient outcomes. Currently, ~90% of children with B-ALL are cured by therapy that includes 12–30 months of low-intensity maintenance chemotherapy, consisting of daily oral mercaptopurine and weekly oral methotrexate, with or without monthly pulses of a corticosteroid plus vincristine. Studies have demonstrated that failure to achieve therapeutic serum concentrations of mercaptopurine, owing to non-compliance or impaired bioavailability, negatively affects survival[10]. To improve mercaptopurine bioavailability, patients have been instructed for decades to take mercaptopurine in the evening without food or dairy products (1–2 h after a meal). Landier *et al.*[10] investigated the association between mercaptopurine ingestion habits and adherence, the risk of relapse, and red-cell thioguanine nucleotide (TGN) levels as a measure of mercaptopurine exposure. Their findings, published in 2017, reveal no difference in relapse risk or TGN levels based on the timing or dietary context of daily drug ingestion. By contrast, these practice restrictions are likely to reduce treatment adherence. Thus, survival outcomes might be improved in some patients by simply eliminating these non-evidence-based guidelines on drug administration.

2017 was a groundbreaking year in ALL therapy: the approval of three different therapies in a single year is unlikely to be repeated. Continued work is needed, however, as none of the approved therapies is a panacea, and a number of questions exist, including how to prioritize the different choices for individual patients, which agents can serve as stand-alone treatments (rather than a bridge to HSCT), and which therapies can be moved into frontline treatment. More work is also required to understand the toxicities of these therapies and the mechanisms of resistance — immunotherapies that target different or multiple surface antigens need to be translated into the clinic as a possible means to circumvent relapse. The advances made in 2017 also remind us that an improved understanding of ALL biology and the importance of treatment adherence might be equally as valuable as groundbreaking new therapies.

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LUNG CANCER IN 2017

Giant steps and stumbling blocks

David F. Heigener and Martin Reck

In 2017, major advances in the treatment of non-small-cell lung cancer (NSCLC) continued to emanate from the fields of molecularly targeted therapy and immunotherapy. In the former, new drugs with improved efficacy and reduced toxicity entered the clinic; in the latter, immune-checkpoint inhibition proved efficacious after chemoradiotherapy for stage III disease, but had disparate results in the frontline treatment of stage IV disease.

Thoracic oncologists have been spoiled by the successful development of effective immunotherapies and molecularly targeted treatments for non-small-cell lung cancer (NSCLC). The current standard-of-care therapies set a high bar for novel treatments and, in 2017, the most important studies with practice-changing results were again predicated on the precision medicine and immunotherapy paradigms.

Most patients with *EGFR*-mutant NSCLC derive substantial clinical benefit from first-generation or second-generation EGFR tyrosine-kinase inhibitors (TKIs), such as erlotinib, gefitinib, and afatinib[1]. However, resistance to these agents invariably develops, and in ~50% of patients is attributable to secondary *EGFR* T790M mutation. Osimertinib, a third-generation EGFR TKI, was developed to overcome this resistance mechanism by specifically and irreversibly inhibiting EGFR variants harbouring the T790M mutation and primary activating mutations. Notably, this compound has virtually no affinity for wild-type EGFR. In the phase III AURA3 trial[2], osimertinib was compared with standard platinum and pemetrexed chemotherapy in 419 patients with a confirmed $EGFR^{T790M}$ mutation after failure of treatment with erlotinib, gefitinib, or afatinib. Results of this trial reported in 2017 demonstrated that, congruent with a marked increase in the objective response rate (ORR), osimertinib prolonged the investigator-assessed median progression-free survival (PFS; the primary end point) by 5.7 months, and by almost 4 months in the subgroup of patients with CNS metastases at baseline[2] (TABLE 1). Importantly, osimertinib also had a better safety profile than chemotherapy, with a lower risk of severe adverse events[2] (TABLE 1). Accordingly, osimertinib became the first FDA-approved targeted therapy for patients with $EGFR^{T790M}$-mutant NSCLC after the failure of standard first-line EGFR-TKI therapy. In the near future, discussions about the optimal position of osimertinib will intensify. This debate has been fuelled by preliminary results of the phase III FLAURA trial[3], presented in 2017, which revealed the superior efficacy and safety of osimertinib versus erlotinib or gefitinib in the frontline treatment of NSCLC with activating *EGFR* mutations (median PFS 18.9 months versus 10.2 months; incidence of grade ≥3 toxicities 34% versus 45%).

ALK fusion proteins, typically resulting from *EML4–ALK* translocations, are key oncogenic drivers in a small subset of patients with advanced-stage NSCLC. In this group, the first-generation ALK TKI crizotinib, an agent initially developed as a MET inhibitor, has been the standard-of-care therapy for the past 5 years[4]. However, patients often have disease progression after a few months of treatment, especially with the occurrence of CNS metastases. These events are probably related to the affinity of crizotinib for multidrug resistance protein 1 (MDR1), a transmembrane protein that can export the drug out of the CNS[5]. Moreover, a range of acquired resistance mutations in the ALK kinase domain render crizotinib ineffective. Thus, second-generation ALK TKIs, such as alectinib, which are not substrates of MDR1 and have proven preclinical activity against cells with *ALK* resistance mutations, might be of greater clinical benefit.

In the phase III ALEX trial[6], alectinib was compared with crizotinib as first-line treatments for 303 patients with ALK-positive NSCLC. After a median follow-up period of 17.6 months with crizotinib and 18.6 months with alectinib, use of the latter drug significantly prolonged investigator-assessed PFS, which was the primary end point[6] (TABLE 1). Notably, 12% of patients treated with alectinib had CNS progression versus 45% of those who received

Table 1 | **Key phase III trials of NSCLC therapies with results reported in 2017**

Trial	Setting	Comparison	ORR	Median PFS (HR, 95% CI)	Grade ≥3 AE rate	Other findings (HR, 95% CI)
AURA3 (REF. 2)	$EGFR^{T790M}$ -mutant stage IV NSCLC after first-line EGFR TKI	Osimertinib vs chemotherapy	71% vs 31% ($P<0.001$)	10.1 mo. vs 4.4 mo. (0.3, 0.23–0.41; $P<0.001$)	23% vs 47%	Median PFS in those with CNS metastases: 8.5 mo. vs 4.2 mo. (0.32, 0.21–0.49)
ALEX[6]	First-line treatment of *ALK*-positive stage IV NSCLC	Alectinib vs crizotinib	82.9% vs 75.5% ($P=0.09$)	NR vs 11.1 mo.; 12-mo. PFS 68.4% vs 48.7% (0.47, 0.34–0.65; $P<0.0001$)	41% vs 50%	Incidence of CNS progression: 12% vs 45% (0.16, 0.10–0.28; $P<0.001$)
CheckMate 026 (REF. 8)	First-line treatment of stage IV NSCLC (with >5% PD-L1 positivity)	Nivolumab vs chemotherapy	26% vs 33% (OR 0.70, 95% CI 0.46–1.06)	4.2 mo. vs 5.9 mo. (1.15, 0.91–1.45; $P=0.25$)	18% vs 51%	• Median OS: 14.4 mo. vs 13.2 mo. (1.02, 0.80–1.30) • TMB was predictive of PFS
PACIFIC[10]	Stage III disease (all subtypes), after CRT	Durvalumab vs placebo	28.4% vs 16.0% ($P<0.001$)	16.8 mo. vs 5.6 mo. (0.52, 0.42–0.65; $P<0.001$)	29.9% vs 26.1%	Median time to metastasis or death: 23.2 mo. vs 14.6 mo. ($P<0.001$); OS data pending

AE, adverse event; CNS, central nervous system; CRT, chemoradiotherapy; mo., months; NSCLC, non-small-cell lung cancer; NR, not reached; OR, odds ratio; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death 1 ligand 1; PFS, progression-free survival; TKI, tyrosine-kinase inhibitor; TMB, tumour mutational burden.

Key advances

- Osimertinib became a new standard of care for patients with non-small-cell lung cancer (NSCLC) who develop *EGFR*^{T790M}-mediated resistance to a first-generation or second-generation EGFR inhibitor[2], and might soon supplant these agents in the frontline[3], based on data reported in 2017
- In patients with *ALK*-positive NSCLC, alectinib — an approved second-line therapy — has been shown to be superior to frontline crizotinib, with impressive efficacy against CNS metastases[6]
- Immune-checkpoint inhibition with the anti-PD-1 antibody nivolumab was not found to be superior to first-line chemotherapy in patients with PD-L1-positive NSCLC; however, tumour mutational burden was identified as a potential biomarker for predicting efficacy in this context[8]
- The anti-PD-L1 antibody durvalumab became the first immune-checkpoint inhibitor with proven efficacy in the consolidation treatment of stage III NSCLC, following chemoradiotherapy[10]

crizotinib[6], strongly supporting the favourable CNS activity of the second-generation TKI. Moreover, severe adverse events were less frequent with alectinib[6] (TABLE 1).These results, together with the findings of the J-ALEX trial in Japan[7], establish the efficacy of alectinib in untreated patients with NSCLC, which seems to be superior to that reported in pretreated patients. Therefore, similarly to the treatment of *EGFR*-mutant disease, optimizing the treatment sequence is an emergent clinical challenge, particularly in a landscape in which multiple alternative agents are in development.

Moving to the field of immunotherapy, more confusing results came from the CheckMate 026 trial[8], in which frontline immune-checkpoint inhibition with the anti-PD-1 antibody nivolumab was compared with standard platinum-based chemotherapy in patients with NSCLC. In 2016, results of the KEYNOTE-024 trial[9] demonstrated that first-line pembrolizumab, another anti-PD-1 antibody, significantly improved the PFS (median 10.3 months versus 6.0 months; $P < 0.001$) and overall survival (HR 0.6, 95% CI 0.41–0.89; $P = 0.005$) of patients with NSCLC who had PD-L1 expression on ≥50% of tumour cells, in comparison with chemotherapy. No such superiority was demonstrated for nivolumab in CheckMate 026: the median PFS in patients with PD-L1 expression on ≥5% of tumour cells, the primary end point, was 4.2 months versus 5.9 months with chemotherapy; similar trends were seen for ORR and overall survival[8] (TABLE 1). Moreover, no difference in efficacy was demonstrated in an exploratory analysis of PFS among the subgroups with high PD-L1 positivity (≥50%)[8].

In comparison with KEYNOTE-024 (REF. 9), the use of different cut-offs for PD-L1 expression and distinct diagnostic antibodies, in addition to imbalances in patient characteristics between the treatment arms (more patients with >50% PD-L1 positivity in the chemotherapy arm)[8], might have contributed to the conflicting results of CheckMate 026. These findings underscore the paramount importance of appropriate biomarkers for selecting patients to receive these novel therapies. An interesting signal for a potential new predictive biomarker comes from a retrospective analysis of tumour mutational burden (TMB) in the CheckMate 026 dataset[8]. In a subset of 312 patients who had tumour and blood samples available for mutational analysis (58% of the intention-to-treat population), those with a high TMB (≥243 missense mutations) derived greater benefit from nivolumab than from chemotherapy, in terms of both the ORR (47% versus 28%) and PFS (median 9.7 months versus 5.8 months)[8]. Patients with a medium or low TMB (101–242 and <100 missense mutations, respectively) derived no such benefit: median PFS 3.6 months and 4.2 months, respectively, versus 6.9 months and 6.5 months with chemotherapy[8]. However, an overall survival benefit could not be detected for any patient subgroup. Notably, TMB was not correlated with PD-L1 expression (Pearson's correlation coefficient 0.059)[8]. Further prospective trials are needed to clarify the role of TMB as a predictive biomarker.

Following the promising results in patients with advanced-stage NSCLC, an increasing number of reports have underlined the potential of immunotherapy at earlier stages of the disease. At present, chemoradiotherapy is the cornerstone of treatment for stage III NSCLC. In the phase III PACIFIC trial[10], the role of consolidation immunotherapy was evaluated, with 719 patients randomly assigned (2:1) to receive either the anti-PD-L1 antibody durvalumab or placebo after chemoradiotherapy. The co-primary end points were independently reviewed PFS and overall survival, measured from initiation of the consolidation phase. The study remains blinded pending the overall survival results, but results published in 2017 demonstrate a median PFS of 16.8 months with durvalumab versus 5.6 months with placebo[10]. Corresponding increases in the ORR and median time to metastasis or death with the anti-PD-L1 antibody were also reported[10] (TABLE 1). These results are impressive, but a key issue remains: will more patients be cured with durvalumab, or will the overall survival benefit be nullified by crossover of patients on the control arm to receive immunotherapy after disease recurrence or progression? These questions will be answered when mature overall survival data become available.

In conclusion, in 2017 we continued to make giant steps forwards in the treatment of metastatic NSCLC with driver mutations, and in immunotherapy for locally advanced disease. However, the contradictory results with immune-checkpoint inhibitor monotherapy in first-line treatment of stage IV disease represent a small stumbling block on the way to determining the optimal treatment algorithm.

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Competing interests statement
D.F.H. and M.R. are advisory board members for, and have received honoraria for speaking and reimbursement for travel from Astra Zeneca, BMS, Boehringer Ingelheim, Lilly, MSD, Pfizer, and Roche. Both authors have also received honoraria for speaking from Chugai and Fresenius; D.F.H. is an advisory board member for both companies, and M.R. for Chugai.

UROTHELIAL CANCER IN 2017

Changes in expectations for metastatic urothelial carcinoma

Joaquim Bellmunt and Rosa Nadal

2017 saw the publication of clinical trial data and the approval of new treatment approaches for metastatic urothelial carcinoma. Pembrolizumab is now a well-established treatment for patients with disease progression after cisplatin, with high-level evidence supporting its superiority over second-line chemotherapy. For patients ineligible for cisplatin, atezolizumab and pembrolizumab provide meaningful clinical benefit as frontline therapies.

The treatment landscape of metastatic urothelial carcinoma (mUC) has shifted dramatically in a short period of time. Before 2016, mUC was considered a devastating, almost uniformly fatal disease (12–15 months overall survival (OS) from diagnosis). In 2017, the results of several studies have led to breakthroughs in the treatment of patients with mUC.

KEYNOTE-045 (REF. 1) was the first trial to show clear superiority of immunotherapy over second-line chemotherapy for patients with platinum-resistant mUC. This phase III trial, in which patients received either pembrolizumab or chemotherapy (paclitaxel, docetaxel or vinflunine), was terminated after meeting its co-primary end points: OS and progression-free survival (PFS)[1]. Analysis of the intention-to-treat (ITT) population (542 patients) revealed, strikingly, that pembrolizumab improved OS compared with chemotherapy with a 27% death-risk reduction (TABLE 1). The overall response rate (ORR) was also significantly improved with pembrolizumab, together with a greater duration of response (≥12 months for 68% of responses)[1].

The biomarker potential of a PD-L1 combined positive score (CPS; a measure of the percentage of PD-L1-positive tumour cells and infiltrating immune cells relative to the total number of tumour cells[2]) was evaluated in a subgroups analysis. Patients with a CPS of ≥10% (about one-third) had an even greater reduction in the risk of death with pembrolizumab versus chemotherapy, although the median OS was shorter than that reported for the ITT population (TABLE 1).

Pembrolizumab was better tolerated than chemotherapy, with fewer treatment-related adverse events (TRAEs) of any grade (60.9% versus 90.2%) and grade 3–5 TRAEs (15% versus 49.4%). With the introduction of immune-checkpoint inhibitors into routine clinical settings, awareness of the risk of the unique and distinct spectrum of immune-related adverse events (irAEs) has increased. In KEYNOTE-045, irAEs were more common with pembrolizumab than with chemotherapy. An association between treatment with immune-checkpoint inhibitors and good quality of life (QoL) is increasingly expected; pembrolizumab also proved superior to chemotherapy for this end point[3]. The QoL benefits of immune-checkpoint inhibitors are widely acknowledged but undeniably, the true appeal of such agents is the potential to extend long-term survival. Two updates of the KEYNOTE-045 data corroborated the extension of survival in the ITT population[4,5].

In 2017, following the accelerated approval of four immune-checkpoint inhibitors for mUC (atezolizumab, nivolumab, durvalumab and avelumab), the FDA granted full approval to pembrolizumab in the second-line setting based on the superior OS data from KEYNOTE-045. Pembrolizumab offers a clinical benefit not previously observed, establishing a new benchmark in the second-line setting. This approval is a milestone because pembrolizumab is the first agent shown to improve OS in patients with mUC in the second-line setting, but also because pembrolizumab was superior over chemotherapy in terms of secondary end points.

Around 50% of patients with mUC are unable to receive cisplatin-based therapy for various reasons (mainly impairment in renal function or poor performance status)[6]. Until 2017, these patients usually received carboplatin-based treatment instead, with a median OS of ~9 months. The results of two phase II studies of immune-checkpoint inhibitors in cisplatin-ineligible patients with previously untreated mUC were presented in 2017 (REFS 2,7). The first of these single-arm studies evaluated the effectiveness of atezolizumab in 119 patients; the ORR was 24% and the median OS was 14.8 months[7] (TABLE 1). The median PFS was very modest (2.7 months), and compared unfavourably with historical outcomes of chemotherapy (~6 months). Most responses were durable and occurred similarly across all PD-L1-defined and poor prognosis factor subgroups in predefined analyses. Results of a *post hoc* analysis showed a substantially higher tumour mutational load (an exploratory biomarker) in patients with a response versus those without a response.

In the KEYNOTE-052 trial[2], involving 370 patients, the response rate to pembrolizumab was 24%, including 5% of patients with a complete response (TABLE 1). Remarkably, 83% of the responses were ongoing at the time of analysis, although at a median follow-up duration of only 5 months. More responses were reported in the CPS ≥10% than in the CPS <10% population (37% versus 18%).

Key advances

- Pembrolizumab is associated with longer overall survival, better quality of life, and fewer adverse events than chemotherapy in patients with metastatic urothelial carcinoma (mUC) with disease progression following cisplatin-based chemotherapy[1]
- Atezolizumab and pembrolizumab substantially improve response rates and overall survival outcomes in patients with mUC who are not eligible for treatment with cisplatin[2,7]
- Tumour angiogenesis presents clear therapeutic opportunities in patients with mUC; however, the addition of ramucirumab to docetaxel offers only a modest level of benefit[9]

Table 1 | **Summary of selected clinical trials in mUC published in 2017**

Trial and refs	Intervention	ORR	Median DoR	Median PFS	Median OS	Practice points
Second-line setting and beyond in patients previously treated with platinum-based therapy						
KEYNOTE-045 (REF. 1)	Pembrolizumab versus chemotherapy (paclitaxel, docetaxel or vinflunine)	21.1% versus 11.4%; $P = 0.001$	NR versus 4.3 mo.	2.1 mo. versus 3.3 mo.; $P = 0.42$	• In ITT population: 10.3 mo. versus 7.4 mo. (HR 0.73; 95% CI 0.59–0.91; $P = 0.002$) • In patients with CPS ≥10%: 8.3 mo. versus 5.2 mo. • (HR 0.57; 95% CI 0.37–0.88; $P = 0.005$)	• Fewer adverse events and superior QoL in pembrolizumab arm
RANGE[9]	Ramucirumab plus docetaxel versus docetaxel	24.5% versus 14%	NR	4.1 mo. versus 2.8 mo. (HR 0.75; 95% CI 0.6–0.94; $P = 0.012$)	NR	• Previous treatment with immune-checkpoint inhibitors allowed • No unexpected toxicities • No differences in QoL
First-line setting in cisplatin-ineligible patients						
IMvigor 210 (cohort 1)[7]	Atezolizumab	23%	NR	2.7 mo. (2.1–4.2 mo.)	15.9 mo. (1.4 mo.–not estimable)	• Common TRAEs: fatigue (30%), diarrhoea (12%) and pruritus (11%) • IrAEs (12%)
KEYNOTE-052 (REF. 2)	Pembrolizumab	ITT: 24% CPS ≥10%: 38%	NR	2 mo. (2–3 mo.)	6-mo. OS: 67% (62–73%)	• Common TRAEs: fatigue (23%), pruritus (15%) and rash (10%)

CPS, combined positive score; DoR, duration of response; irAE, immune-related adverse event; ITT, intention to treat; mUC, metastatic urothelial carcinoma; mo., month(s); NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; QoL, quality of life; TRAE, treatment-related adverse-events.

Atezolizumab and pembrolizumab received conditional regulatory approval in the first-line setting in 2017. As such, immune-checkpoint inhibitors will challenge chemotherapy as a frontline treatment option in cisplatin-ineligible patients with mUC. To date, no prospective comparison of frontline treatments has been completed; the role of immune-checkpoint inhibitors in this setting needs to be defined.

A key limitation of agents targeting the PD-1–PD-L1 axis is the sobering observation that only 20–24% of patients with mUC are likely to benefit from them. Major improvements are needed; agents targeting tumour angiogenesis have shown promise in various cancers. The first evidence of the potential of antiangiogenic therapy in patients with mUC came from a randomized phase II trial of the anti-VEGFR2 antibody ramucirumab combined with docetaxel[8]. In the phase III RANGE study[9], 530 patients with platinum-resistant mUC received docetaxel with either ramucirumab or placebo. Previous treatment with one immune-checkpoint inhibitor was allowed; data were only reported for 14 patients in this situation[9], thus limiting their interpretation. The reported analysis was based on investigator-assessed PFS and ORRs in the first 437 patients of the ITT population. Patients treated with ramucirumab had a significantly longer median PFS and ORR[9] (TABLE 1). A 'gatekeeping design' was implemented and thus, a superior ORR could not be formally tested because no benefit in OS was demonstrated at the time of analysis. The safety data[9] revealed no unexpected toxicities. The mean scores for global QoL were mostly unchanged over time, but these outcomes were reported after a median follow-up duration of 5 months. Despite the lack of mature survival data (not a primary end point), a PFS benefit of only 1.3 months was reported, tempering the initial enthusiasm for this combination. With the increasing availability of immune-checkpoint inhibitors, these results should be interpreted in the context of a shift in expectations for at least a minority of patients with mUC. Nevertheless, ramucirumab might be indicated as a third-line treatment option after immune-checkpoint inhibition as the trial progresses and survival data become available.

In the future, we hope to see the development of meaningful predictive biomarkers and the integration of immunotherapy into the paradigm of cytotoxic chemotherapy, and new combinations with other immune-checkpoint inhibitors and targeted agents.

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Competing interests statement

J.B. has been a consultant for Agensys, Amgen, Astra Zeneca, Bayer, Eisai, Genentech, Janssen, Merck, Novartis, Pfizer, Sanofi, and Seattle Genetics; has received financial support for research from Novartis and Sanofi, and royalties for his UpToDate contribution in urothelial tumours. R.N. declares no competing interests.

MELANOMA IN 2017

Moving treatments earlier to move further forwards

Michael A. Davies and Keith T. Flaherty

In 2017, results from phase III trials demonstrated the impressive safety and efficacy of adjuvant targeted and immune therapies in patients with resectable stage III–IV melanoma, and raised questions about the surgical management of patients with microscopic sentinel-lymph-node metastases. For patients with unresectable disease, new overall survival data added to the debate about the relative benefits of single-agent anti-PD-1 versus combined anti-PD-1 and anti-CTLA-4 immunotherapy.

The treatment of metastatic melanoma has been transformed by the approval of 10 new targeted and immune therapies between 2011 and 2016 (REF. 1). No new approvals have yet been granted in 2017, but the year has been punctuated by results that will further affect treatment decisions for patients with stage IV melanoma, and will probably also transform the care of those with stage III disease.

In 2015, the anti-CTLA-4 antibody ipilimumab was approved by the FDA for the adjuvant treatment of patients with resected stage III melanoma based on the results of the EORTC 18071 trial[2]. Ipilimumab was associated with improved relapse-free survival (RFS), distant-metastasis-free survival (DMFS), and overall survival (OS) compared with placebo, but >50% of patients developed grade 3–4 adverse events (AEs), and five patients (1.1%) died as a result of autoimmune toxicities[2,3]. This level of risk might be unacceptable to many patients with stage III disease, considering that surgery alone is often curative, and particularly if safer treatments become available.

In the CheckMate 238 study[4], patients with resectable stage IIIB, IIIC, or IV melanoma were randomly assigned (1:1) to receive 1 year of adjuvant immunotherapy with either the anti-PD-1 antibody nivolumab or ipilimumab, starting within 12 weeks after complete tumour resection and completion lymph-node dissection (CLND); the primary end point was RFS. In 2017, the first mandated interim analysis, at a follow-up duration for all patients of ≥18 months after randomization, was reported[4]. Nivolumab was superior to ipilimumab (similar to previous results in the setting of stage IV disease), with a 12-month RFS of 70.5% versus 60.8%[4] (TABLE 1). Subset analyses revealed the superiority of nivolumab across almost all patient subgroups, including those with PD-L1-positive and PD-L1-negative tumours[4]. Nivolumab also improved DMFS (HR 0.73, 95% CI 0.55–0.95)[4]; however, no OS outcomes were reported. Importantly, nivolumab was also safer than ipilimumab, as expected, with markedly lower rates of grade 3–4 AEs (14.4% versus 45.9%) and treatment discontinuation owing to AEs (9.7% versus 42.6%)[4].

Initial results of the COMBI-AD trial[5] were released simultaneously with those of CheckMate 238 (REF. 4). In COMBI-AD[5], patients with stage III melanoma harbouring a *BRAF*$^{V600E/K}$ mutation, the most common oncogenic aberration in cutaneous melanomas (~50%), were randomly assigned (1:1) to receive either 1 year of adjuvant targeted therapy with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib (D + T) or placebo, again starting within 12 weeks of complete resection and CLND. After a minimum follow-up duration of 2.5 years, the primary end point of an improvement in RFS with D + T was met[5] (TABLE 1). The estimated 3-year RFS was 58% with D + T versus 39% with placebo (HR 0.47, 95% CI 0.39–0.58; $P < 0.001$). Importantly, D + T also improved OS (HR 0.57, 95% CI 0.42–0.79; $P = 0.0006$), although this result did not cross the pre-specified boundary for statistical significance ($P = 0.0000019$). The safety profile of adjuvant D + T was similar to that reported in patients with stage IV disease, with 36% of patients having grade 3–4 AEs, and 26% discontinuing treatment owing to toxicities.

These results suggest that nivolumab and D + T, although not yet approved by the FDA, will become standard adjuvant therapies for stage III melanoma in the near future. These therapies have been shown to improve OS in patients with stage IV disease, and the improvement in OS detected in COMBI-AD indicates that the survival of patients with melanoma might be further improved by using active agents earlier in the course of disease. Notably, the results from these studies do not enable direct comparisons of the efficacy of adjuvant nivolumab and D + T, owing to eligibility criteria that encompassed different disease stages, thus invalidating comparisons of landmark data, and the use of different comparator arms, which prevents comparisons of hazard ratios. Thus, the best approach to choosing between adjuvant targeted and immune therapies for individual patients will be debated, similar to the current discussions on the optimal management strategy for stage IV disease[1]. Furthermore, new questions will soon arise regarding the appropriate management of patients with disease relapse after the use of these adjuvant treatments.

Moving forward, the interpretation of the data from these trials and ongoing adjuvant studies might be complicated by changes in the surgical management of patients with

Key advances

- New data from phase III trials suggest that sweeping changes in the management of melanoma patients with resectable regional metastases are imminent, with both anti-PD-1 immunotherapy[4] and combined BRAF and MEK inhibition[5] showing promising efficacy in the adjuvant setting
- By contrast, immediate completion lymph-node dissection provided no benefit compared with surveillance in patients with resectable, sentinel-lymph-node-positive melanoma[7]
- Data published in 2017 also further inform the continuing discussion of the relative risks and benefits of single-agent anti-PD-1 immunotherapy versus combination anti-PD-1 and anti-CTLA-4 immunotherapy for patients with unresectable disease[9]

Table 1 | **Key phase III trials of melanoma therapy with results published in 2017**

Trial	Study population	Interventions	Primary end point	Result (HR, 95% CI)
CheckMate 238 (REF. 4)	Patients with surgically resected, stage IIIB–C or IV disease ($n = 903$)	Adjuvant nivo vs ipi	RFS	1-year RFS: 70.5% vs 60.8% (0.65, 0.51–0.83; $P < 0.001$)
COMBI-AD[5]	Patients with surgically resected stage IIIA–C disease* ($n = 870$)	Adjuvant dabrafenib + trametinib vs placebo	RFS	1-year RFS: 88% vs 56% (0.51, 0.40–0.65; $P < 0.001$)
MSLT-II[7]	Patients with positive SLNB ($n = 1,939$)	Immediate CLND vs surveillance (CLND upon nodal recurrence)	MSS	3-year MSS: 86% vs 86%; $P = 0.42$
CheckMate 067 (REF. 9)	Patients with untreated stage IV or unresectable stage III disease ($n = 945$)	Ipi vs nivo vs ipi + nivo	OS	3-year OS: 34% with ipi vs 52% with nivo vs 58% with ipi + nivo (nivo vs ipi: 0.65, 0.53–0.80, $P < 0.001$; ipi + nivo vs ipi: 0.55, 0.45–0.69, $P < 0.001$; ipi + nivo vs nivo: 0.85, 0.68–1.07)

CLND, completion lymph-node dissection; ipi, ipilimumab; MSS, melanoma-specific survival; nivo, nivolumab; OS, overall survival; RFS, relapse-free survival; SLNB, sentinel-lymph-node biopsy. *Patients with stage IIIA disease had to have >1 mm tumour deposits in at least one regional lymph node to be eligible.

microscopic lymph-node metastases, who account for ~70% of all patients with stage III melanoma owing to the widespread adoption of the SLN biopsy (SLNB) procedure in those with high-risk primary tumours[6]. Patients with a positive SLNB are generally offered immediate CLND; however, the clinical benefit of this procedure remains unclear. Thus, in the MSLT-II trial[7], 1,939 patients with tumour-positive SLNBs were randomly assigned (1:1) to an immediate CLND group, or to an active-surveillance programme in which CLND was performed only if and when evidence of nodal recurrence was detected clinically. At a median follow-up duration of 43 months, 3-year melanoma-specific survival was 86% for both groups[7] (TABLE 1). Notably, however, patients in the immediate CLND group had a significantly higher risk of lymphoedema (24.1% versus 6.3%; $P < 0.001$)[7], a finding that, in combination with the equivalent survival outcomes, will probably result in a marked reduction in the use of this procedure. While such a change in practice is well supported by the results of this trial, involvement of non-SLNs among patients in the immediate CLND group predicted a much higher risk of melanoma-related mortality. Thus, new risk models based on SLN status will need to be developed to guide patient management and the design of future adjuvant trials.

Important new data relating to patients with unresectable advanced-stage disease were also reported in 2017. In the CheckMate 067 study[8,9], treatment-naive patients with unresectable stage III–IV melanoma received either ipilimumab, nivolumab, or both agents. The trial was powered to compare each of the nivolumab-containing arms versus single-agent ipilimumab, although most interest focused on the relative outcomes of patients treated with nivolumab versus ipilimumab plus nivolumab. Initial results reported in 2015 demonstrated that the combination therapy resulted in a higher objective response rate (ORR) and improved progression-free survival compared with either monotherapy, but with markedly greater toxicity[8]. An important update was provided in 2017, which for the first time included OS outcomes[9] (TABLE 1). The data showed that, while the ORR with ipilimumab plus nivolumab was indeed higher than with single-agent nivolumab (58% versus 44%), the difference in OS at 2 years and 3 years was only 5% and 6%, respectively, favouring the combination arm[9]. This smaller difference in OS relative to ORRs probably relates to the fact that ipilimumab has an ORR of ~10% in patients with progressive disease after anti-PD-1 therapy, which is similar to the rate seen in treatment-naive patients[10]; thus, receipt of ipilimumab by some patients after disease progression on nivolumab might have nullified the apparent initial benefit of combination therapy. In an intriguing planned subgroup analysis, 3-year OS was virtually identical in patients with PD-L1 staining on ≥1% of tumour cells (HR 1.02), whereas the combination was superior to nivolumab alone in those with <1% PD-L1 positivity (HR 0.70)[9]. Additional validation is needed, but these results indicate that PD-L1 might be an important biomarker to guide immunotherapy selection. The results also support continued investigation of new combinatorial strategies to improve OS outcomes in patients with stage IV melanoma.

These results confirm 2017 as another landmark year for melanoma research, with the benefit of immune and targeted therapies extending to patients with stage III disease. These findings also highlight the need to develop new biomarkers in order to optimize the management of patients with melanoma.

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M.A.D. has served on advisory boards for BMS, Novartis, Roche/Genentech, Sanofi-Aventis, and Vaccinex, and has been the Principal Investigator of studies funded by grants to his institution from AstraZeneca, BMS, Oncothyreon, Roche/Genentech, and Sanofi-Aventis. K.T.F. has served on advisory boards for BMS, Merck, Novartis, and Roche/Genentech, and has received grant support from Novartis.

COLORECTAL CANCER IN 2017

Practice-changing updates in the adjuvant and metastatic setting

Alberto Puccini and Heinz-Josef Lenz

2017 has been full of new discoveries that will influence the treatment of colorectal cancer. In the adjuvant setting, 3 months of chemotherapy might now be considered a new standard of care. Various other new treatments and promising biomarkers have also become available that will improve survival outcomes and the quality of life of many patients with metastatic disease.

Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide. In the past decade, numerous exciting advances have been made in the treatment of patients with metastatic disease. In fact, since bevacizumab was approved for patients with metastatic CRC (mCRC) in 2004, a further six agents (both biologic and chemotherapeutic compounds) have become standard-of-care treatments. Along with improvements in surgical and interventional radiology techniques, in the management of toxicities and in palliative care, the median overall survival of patients with mCRC can now be prolonged for >30 months. However, an unmet need for new treatments and biomarkers remains.

Following the publication of data from the MOSAIC study in 2004, which demonstrated the efficacy of oxaliplatin-based adjuvant chemotherapy in patients with stage III colon cancer, no major treatment advances have been made in the adjuvant setting. Moreover, only 20% of patients with resected stage III colon cancer, and only 5–10% with high-risk stage II disease really benefit from adjuvant treatment, meaning that the majority of patients are exposed to unnecessary toxicities, mostly owing to cumulative and potentially long-lasting oxaliplatin-related neurotoxicities.

The International Duration Evaluation of Adjuvant Chemotherapy (IDEA) collaboration was designed to determine whether the duration of oxaliplatin-based adjuvant chemotherapy can be decreased from 6 months, the current global standard of care, to 3 months without compromising efficacy, while also improving both tolerability and costs. In this collaboration, investigators performed a prospective, preplanned pooled analysis of data from six randomized phase III trials designed to assess the non-inferiority of 3 months of adjuvant chemotherapy with either 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX) or capecitabine and oxaliplatin (XELOX) compared with 6 months of either regimen in patients with stage III colon cancer. The results were presented at the ASCO Annual Meeting 2017 (REF. 1). This huge endeavour assembled data from nearly 13,000 patients enrolled between 2007 and 2015 in studies in 12 countries: TOSCA (Italy, the first trial to start enrolling patients in 2007), SCOT (UK, Denmark, Spain, Australia, Sweden, New Zealand), Alliance/SWOG 80702 (USA, Canada), IDEA (France), ACHIEVE (Japan), and HORG (Greece). In order to determine non-inferiority, the predefined two-sided 95% CI for disease-free survival (DFS), which was the primary end point of the study, needed to be <1.12. Overall, 3-year DFS was 74.6% in the 3-month arm and 75.5% in the 6-month arm (HR 1.07, 95% CI 1.00–1.15). Thus, from a purely statistical point of view, non-inferiority was not established, suggesting that 3 months of treatment are not as beneficial as 6 months. However, the DFS curves overlap and the absolute gain in efficacy with 6 months of treatment is <1%, while the risk of severe (grade ≥3) neurotoxicities was much higher in the 6-month arm versus the 3-month arm (16% versus 3% with FOLFOX, 9% versus 3% with XELOX; P <0.0001 for both comparisons). In addition, in the preplanned subgroup analysis, non-inferiority was established in patients who received XELOX (HR 0.95, 95% CI 0.85–1.06), but not in those treated with FOLFOX (HR 1.16, 95% CI 1.06–1.26), and in patients with T1–3N1 disease (HR 1.01, 95% CI 0.90–1.12), but not in those with T4 or N2 disease (HR 1.12, 95% CI 1.03–1.23). On the basis of these data, the IDEA trial is leading to a paradigm shift in the adjuvant treatment of patients with stage III colon cancer. These results have created several controversies and have several shortcomings; although, despite the fact that the trial failed its primary end point, the differences in DFS are so limited, and the differences in toxicity so much better, that 3 months of oxaliplatin-based chemotherapy should be considered the standard of care for patients with T1–3 N1 stage III colon cancer. However, in patients with a higher risk of disease relapse (those with T4 or N2 disease), oncologists should continue to plan 6 months of adjuvant chemotherapy.

In the past few years, the introduction of immune-checkpoint inhibitors has dramatically improved the standard of care for many types of cancer. Nevertheless, CRC was originally shown to be resistant to this therapy in various studies. In the past 2 years, the presence of DNA mismatch repair deficiency (dMMR) has been proved to strongly predict sensitivity to immune-checkpoint inhibition in a variety of solid tumours, including mCRC[2,3]. This finding has led to the accelerated approval of pembrolizumab, an anti-PD-1 antibody, for the treatment of patients with unresectable or metastatic microsatellite-instability-high (MSI-H)/dMMR solid tumours in May 2017. This is the first example of FDA approval of an anticancer treatment based on the presence of a biomarker, rather than on the body location from which the tumour originated. Moving from these data, CheckMate 142, an open-label, multicentre, phase II clinical trial, was designed to assess the efficacy of nivolumab, an anti-PD-1 antibody, or nivolumab plus ipilimumab, an anti-CTLA-4 antibody, in patients with MSI-H or non-MSI-H mCRC. Results obtained using nivolumab monotherapy in patients with MSI-H mCRC in CheckMate 142 were reported in 2017 (REF. 4). A total of 74 patients with MSI-H/dMMR mCRC received nivolumab (3 mg/kg every 2 weeks) until disease progression, death, unacceptable adverse effects,

Key advances

- On the basis of results of the IDEA trial, 3 months, compared with the current standard-of-care 6 months, of adjuvant chemotherapy for patients with stage III colon cancer dramatically decreases the risk of neurotoxicities without compromising overall efficacy[1]
- Immune-checkpoint inhibitors targeting PD-1 (pembrolizumab and nivolumab) were approved in 2017 by the FDA for microsatellite instability-high DNA mismatch repair deficient metastatic colorectal cancer, thus providing further treatment options for these patients[2,4]
- Tumour sidedness is an emerging and promising biomarker with both prognostic and predictive value that can influence the choice of biologic agent (anti-VEGF versus anti-EGFR antibodies) for first-line treatment of patients with *RAS*-wild-type metastatic colorectal cancer[5–7]

or withdrawal from the study. The overall investigator-assessed objective response rate (ORR) was 31.1% at a median follow-up duration of 12 months, while 69% of patients had disease control for ≥12 weeks. Remarkably, eight (out of 23) responders had responses lasting ≥12 months, which is even more impressive considering that 54% of patients enrolled had received at least three previous treatments. On the basis of these results, on the 31st of July 2017, the FDA granted accelerated approval to nivolumab for the treatment of patients aged ≥12 years with MSI-H/dMMR mCRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.

These data are astonishing, although only a few patients with mCRC will benefit from these treatments. In fact, only 5% of all patients with mCRC have MSI-H/dMMR disease, meaning that almost 95% of patients are considered resistant to immune-checkpoint inhibition. For this reason, both new agents and new biomarkers are urgently needed to improve the overall survival outcomes in the majority of patients.

Beyond MSI-H/dMMR status, evidence continues to mount that tumour sidedness (right versus left) is a promising prognostic and predictive biomarker in patients with mCRC. In fact, data from studies involving tumours arising from different sides of the colon have revealed variations in molecular characteristics and clinical outcomes, which might reflect different embryological origins. Arnold *et al.*[5] investigated the prognostic and predictive relevance of the side of the primary tumour in 2,159 patients with unresectable *RAS*-wild-type mCRC enrolled in six randomized trials (CRYSTAL, FIRE-3, CALGB 80405, PRIME, PEAK, and 20050181) designed to compare the efficacy of chemotherapy plus anti-EGFR antibodies versus that of chemotherapy with or without bevacizumab. A significantly worse prognosis was observed in patients with right-sided tumours than in those with left-sided tumours in both the pooled experimental and control arms for overall survival (HR 2.03, 1.38, respectively; $P < 0.001$), progression-free survival (PFS; HR 1.59; $P < 0.001$, and HR 1.25; $P = 0.008$), and ORR (OR 0.38, 0.56; $P < 0.001$). In addition, right-sided tumour location was not associated with a significant level of benefit from treatment with anti-EGFR antibodies in terms of either overall survival (HR 1.12; $P = 0.38$) or PFS (HR 1.12; $P = 0.37$) compared with a significant benefit from anti-EGFR antibodies, which was observed in patients with left-sided tumours in terms of both overall survival (HR 0.75; $P < 0.001$) and PFS (HR 0.78; $P < 0.001$). Owing to the retrospective nature of this analysis, these data should be interpreted with caution. However, these findings are consistent with those of two other studies published in the same period[6,7], thus increasing the external validity of these data.

Tumour sidedness seems to be a surrogate for genetic differences that drive the development and progression of colon cancers arising in different locations. In 2015, a new consensus molecular subtypes (CMS) classification divided CRC into four distinct subgroups: microsatellite-instability immune (CMS1), canonical (CMS2), metabolic (CMS3), and mesenchymal (CMS4) — each characterized by specific pathway enrichment traits[8]. In 2017, in a retrospective analysis of data from 581 patients with *RAS*-wild-type disease enrolled in the CALGB/SWOG 80405 trial, researchers confirmed the prognostic value of the CMS classification. In addition, they were able to demonstrate that patients with CMS1 colon cancer, which is more frequently diagnosed in the right side of the colon, benefit more from bevacizumab-based treatment than from cetuximab-based treatment (median overall survival 22.5 months versus 11.7 months; $P = 0.029$); CMS2, which is more common in patients with left-sided CRCs, is associated with the opposite sensitivity profile[9]. Further prospective analyses are warranted to validate this potentially practice-changing biomarker. However, these data are consistent across various studies, providing a sound rationale for the implementation of anti-EGFR antibodies in patients with left-sided *RAS*-wild-type mCRC, whereas bevacizumab should be preferred for those with right-sided *RAS*-wild-type mCRC.

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BREAST CANCER IN 2017

Spurring science, marking progress, and influencing history

Jose Perez-Garcia and Javier Cortes

Data published in 2017 underscore the benefit of optimizing anti-HER2 therapy in early stage high-risk HER2-positive disease, and of capecitabine in patients with residual disease after optimal neoadjuvant therapy. In the advanced-stage setting, endocrine therapy combined with cyclin-dependent kinase 4/6 inhibitors, or olaparib could become the preferred option.

Over the past few years, tremendous progress has been made in anticancer therapies; however, the common procedure before considering a new strategy as practice changing is to wait until complete manuscripts are published. In 2017, results from some of the most eagerly awaited studies on breast cancer have been published, encompassing realistic and positive consequences for both patients and society in two different ways: by improving the prognosis of patients with breast cancer, and by potentially decreasing the cost of treatments.

Neoadjuvant therapy for patients with early-stage breast cancer has undergone a continuous evolution. Classical benefits of this approach included tumour downstaging, increasing use of breast-conserving surgery, and the possibility of *in vivo* assessment of therapeutic efficacy. In the past few years, two important new implications for the use of neoadjuvant therapy have been introduced: the accelerated approval of new drugs on the basis of pathological complete response (pCR) as a surrogate end point, and the possibility of a second therapeutic chance when a good response to neoadjuvant therapy is not achieved.

Pertuzumab, an inhibitor of HER2 dimerization, was the first drug to receive accelerated approval for the neoadjuvant treatment of HER2-positive early-stage breast cancer on the basis of the statistically significant and consistent improvement of pCR rates achieved when this agent was added to trastuzumab plus chemotherapy, compared with the addition of placebo to this combination. This approval was conditional on the long-term outcomes of the APHINITY trial[1], a phase III study involving 4,805 women with HER2-positive breast cancer, in which the addition of pertuzumab to adjuvant standard-of-care trastuzumab plus chemotherapy was evaluated (median follow-up duration 45.4 months). The chances of invasive breast cancer recurrence were 19% lower when pertuzumab was added to trastuzumab plus chemotherapy, compared with placebo (HR 0.81, 95% CI 0.66–1; $P=0.045$); the benefit from pertuzumab was slightly greater for patients with node-positive and hormone-receptor-negative disease. Of note, the separation of the invasive disease-free survival curves between the third and fourth year of treatment is interesting, confirming that these data are immature. Nevertheless, this trial is, in our opinion, of critical importance for two reasons: first, these data support the neoadjuvant model as a valid platform for accelerated approval of new drugs in patients with early stage breast cancer; and second, pertuzumab clearly improves the outcomes of patients with resected high-risk HER2-positive breast cancer.

The possibility of a second opportunity for patients without an optimal response to neoadjuvant therapy has guided the design of clinical trials for this patient population. The CREATE-X trial[2] assessed the addition of adjuvant capecitabine versus placebo to standard chemotherapy in 910 patients of Asian origin with HER2-negative breast cancer who had residual disease after receiving standard neoadjuvant chemotherapy. In the final analysis, disease-free survival (HR 0.7, 95% CI 0.53–0.92; $P=0.01$) and overall survival (HR 0.59, 95% CI 0.39–0.9; $P=0.01$) were longer in the capecitabine group than in the control group; patients with hormone-receptor-negative disease derived a greater benefit from the addition of capecitabine (disease-free survival 69.8% versus 56.1% with placebo). Despite some concerns regarding the increased sensitivity of patients of Asian ethnicity to fluoropyrimidines[3], the use of adjuvant capecitabine has been adopted worldwide in clinical practice. Of interest, ongoing trials are evaluating the benefit of other adjuvant therapies in patients with a poor prognosis without a good response to neoadjuvant therapy.

The most important practice-changing studies published in 2017 were conducted in the advanced-stage disease setting. In the past few years, third-generation aromatase inhibitors and high-dose fulvestrant have been the preferred first-line and second-line therapeutic options, respectively, for patients with postmenopausal oestrogen receptor (ER)-positive/HER2-negative metastatic breast cancer (mBC). In combination with cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, these drugs have shown greater antitumour activity than when given as monotherapy, leading to a change in clinical management. Following the publication of the results obtained with the first two CDK4/6 inhibitors, palbociclib and ribociclib, data on a third CDK4/6 inhibitor, abemaciclib, were eagerly expected. The MONARCH 2 study[4] was a phase III trial in which 669 patients with ER-positive/HER2-negative mBC and disease progression after endocrine therapy were randomly assigned to receive fulvestrant plus either abemaciclib or placebo. The addition of abemaciclib significantly increased

Key advances

- The addition of pertuzumab to adjuvant trastuzumab plus chemotherapy improved invasive disease-free survival outcomes in patients with resected high-risk HER2-positive breast cancer[1]
- In patients with a poor prognosis and no response to neoadjuvant therapy, the addition of capecitabine to standard adjuvant chemotherapy is associated with improved outcomes[2]
- In patients with metastatic breast cancer, the addition of cyclin-dependent kinase 4/6 inhibitors to endocrine therapy improves progression-free survival (PFS) outcomes[4,5]
- In patients with metastatic breast cancer harbouring germline *BRCA* mutations, treatment with olaparib is associated with better PFS outcomes than standard chemotherapy[6]
- Trastuzumab biosimilars are becoming a realistic option for HER2-positive breast cancer[8,9]

Table 1 | **Outcomes with CDK4/6-inhibitor-based practice-changing regimens**

Trial and refs.	Study treatments	HR (95% CI)	Median PFS (months)
First-line setting			
PALOMA-2 (REF. 10)	Letrozole + palbociclib versus letrozole + placebo	0.58 (0.46–0.72)	24.8 versus 14.5
MONALEESA-2 (REFS 11,12)	Letrozole + ribociclib versus letrozole + placebo	0.56 (0.43–0.72)	NR (25.3)* versus 14.7 (16)*
MONARCH 3 (REF. 5)	NSAI + abemaciclib versus NSAI + placebo	0.54 (0.41–0.72)	NR versus 14.7
Endocrine-resistant disease			
PALOMA-3 (REF. 13)	Fulvestrant + palbociclib versus fulvestrant + placebo	0.46 (0.36–0.59)	9.5 versus 4.6
MONARCH 2 (REF. 4)	Fulvestrant + abemaciclib versus fulvestrant + placebo	0.55 (0.45–0.68)	16.4 versus 9.3

CI, confidence interval; HR, hazard ratio; NR, not reached; NSAI, non-steroidal aromatase inhibitor (letrozole or anastrozole); PFS, progression-free survival.*Updated data[11].

median progression-free survival (PFS), the primary end point of the study (TABLE 1). A second study, MONARCH 3 (REF. 5), was a phase III trial that included 493 patients with postmenopausal ER-positive/HER2-negative mBC who had not received previous systemic treatment. These patients were randomly assigned to receive letrozole or anastrozole plus either abemaciclib or placebo. The study met its primary end point by demonstrating an improvement in median PFS with the addition of abemaciclib (TABLE 1). These impressive and similar improvements in PFS have important consequences for clinical practice, even in the absence of an overall survival benefit: first, therapy selection will depend on toxicity and/or economic aspects; second, the delay in the use of cytotoxic chemotherapy will improve the quality of life of patients; and third, disease control with orally administered drugs is preferable for patients compared with intravenous drugs, allowing them to forgo or reduce the number of hospital treatments.

For patients with germline *BRCA*-positive mBC, poly [ADP-ribose] polymerase (PARP) inhibitors have shown promising activity and will become available in routine clinical practice in the upcoming months. In the randomized phase III study OlympiAD[6], olaparib monotherapy was compared with physician's choice of chemotherapy in 302 patients with platinum-sensitive HER2-negative mBC with a germline *BRCA* mutation who had received treatment with no more than two chemotherapy regimens. Median PFS was significantly longer with olaparib than with standard therapy (7 months versus 4.2 months; HR 0.58, 95% CI 0.43–0.80; $P < 0.001$), with a favourable toxicity profile. The role of PARP inhibitors in patients with platinum-resistant disease is a matter of debate[7]; the patient subpopulations that will derive optimal levels of benefit from olaparib remain to be determined. The evaluation of PARP inhibitors in patients with homologous recombination deficiencies in the absence of a germline *BRCA* mutation, and/or in the adjuvant setting, could prove relevant.

The current drug-pricing system for new drugs is unsustainable and will only get worse; thus, ensuring the successful integration of biosimilar drugs into the anticancer treatment armamentarium is critical. Several studies have shown comparable efficacy of trastuzumab biosimilars and reference trastuzumab in terms of pCR rates in the neoadjuvant setting[8], or overall response rates in the advanced-stage disease setting[9]. The use of trastuzumab biosimilars is expected to grow worldwide and will enable an increasing number of patients from low-income and middle-income countries to benefit from this agent, but also might reduce the excessively high costs associated with cancer treatment in high-income countries. New combination strategies involving other monoclonal antibodies (such as pertuzumab) should be explored if biosimilar drugs are to become widely used to treat patients with breast cancer.

Despite the advances of the past few years, mBC remains an incurable disease, and the triple-negative subtype is associated with dismal outcomes. Ongoing and future trials of immunotherapies, new targeted agents, antibody–drug conjugates, and new cytotoxic agents will lead to improved management of this disease. We anticipate 2018 to be another year of great success in the search for treatments for cancer in general, and breast cancer in particular.

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MANAGEMENT OF T2DM IN 2017

Clinically relevant results from cardiovascular outcome trials

Rury R. Holman

The risk of death from cardiovascular causes in people with type 2 diabetes mellitus remains around twice that in the general population, with heart failure a common event. In 2017, results from cardiovascular outcome trials in people with diabetes mellitus showed that some drugs have dual utility — reducing cardiovascular risk and improving glycaemic control.

Heart failure and diabetes mellitus frequently coexist, with diabetes mellitus being a major independent risk factor for the progression of heart failure and for the increased risk of both hospital admission for heart failure and premature death, compared with those without diabetes mellitus[1]. The CANVAS Program, which combined data from two trials, included a total of 10,142 people with type 2 diabetes mellitus and high cardiovascular risk who were randomly assigned to receive canagliflozin or a placebo and were followed up for a mean of 3.6 years[2]. In 2017, the CANVAS investigators reported that 14% fewer participants in the canagliflozin group than in the placebo group had a primary composite outcome of cardiovascular death, nonfatal myocardial infarction or nonfatal stroke (26.9 versus 31.5 per 1,000 patient-years, HR 0.86, 95% CI 0.75–0.97, $P = 0.02$). Crucially, hospitalization for heart failure was also reduced by 33% in the canagliflozin group (HR 0.67, 95% CI 0.5–0.87), with a 22% reduction in a composite of death from cardiovascular causes or hospitalization for heart failure (HR 0.78, 95% CI 0.67–0.91). Adverse reactions were broadly consistent with those seen previously with canagliflozin, except that an almost twofold increased risk of amputation was reported, primarily at the toe or metatarsal (6.3 versus 3.4 participants per 1,000 patient-years, HR 1.97, 95% CI 1.41–2.75). This confirmation that the reduction in cardiovascular risk with a sodium–glucose cotransporter 2 inhibitor seems to be a class effect, as also suggested by the CVD-REAL Nordic multinational observational analysis[3], will probably lead to these agents being given greater prominence in guidelines and being used more extensively in populations similar to those studied to date.

Sacubitril/valsartan is a dual-acting angiotensin receptor–neprilysin inhibitor consisting of a 1:1 mixture of valsartan and sacubitril that was evaluated in the prospective comparison of ARNI (angiotensin receptor–neprilysin inhibitor) with ACEI (angiotensin-converting enzyme inhibitor) to determine impact on global mortality and morbidity in heart failure (PARADIGM-HF) trial. The trial coordinators randomly assigned 8,442 patients with class II–IV heart failure and an ejection fraction of ≤40% to either sacubitril/valsartan or enalapril (an ACE inhibitor)[4]. The trial was stopped early, after 2.3 years, as a result of the overwhelming benefit of sacubitril/valsartan, compared with enalapril, on the primary composite outcome of death from cardiovascular causes or hospitalization for heart failure (21.8% versus 26.5%, HR 0.80, 95% CI 0.73–0.87, $P < 0.001$). Importantly, similar benefits were observed in participants with and without diabetes mellitus at entry to the study ($P_{interaction} = 0.40$). All-cause mortality was also 16% lower (overall HR 0.84, 95% CI 0.76–0.93, $P < 0.001$) with sacubitril/valsartan, compared with enalapril. In 2017, a post-hoc analysis of the 3,778 patients with known or previously undiagnosed diabetes mellitus showed a significantly greater mean reduction in HbA_{1c} following 1 year of treatment with sacubitril/valsartan than with enalapril (0.26 ± 1.25% versus 0.16 ± 1.40%)[5]. This benefit persisted during follow-up and was associated with a 23% lower rate of addition of oral antidiabetic agents to a patient's treatment and a 29% lower rate of commencing insulin therapy. These findings demonstrate the dual utility of sacubitril/valsartan with respect to reducing cardiovascular morbidity and mortality as well as improving glycaemic control.

The primary results of the canakinumab anti-inflammatory thrombosis outcome study (CANTOS) were reported in 2017 (REF. 6). In this trial, 10,061 patients (39.9% with diabetes mellitus) who had a previous myocardial infarction and an elevated level of high-sensitivity C-reactive protein were randomly assigned to receive either a placebo or canakinumab (a therapeutic monoclonal antibody targeting IL-1β) at a dose of 50 mg, 150 mg or 300 mg, administered subcutaneously every 3 months. Compared with the placebo, the primary efficacy composite outcome (cardiovascular death, nonfatal myocardial infarction or nonfatal stroke) was lower with all doses of canakinumab (50 mg HR 0.93, 95% CI 0.80–1.07, $P = 0.30$; 150 mg HR 0.85, 95% CI 0.74–0.98, $P = 0.021$; 300 mg HR 0.86, 95% CI 0.75–0.99, $P = 0.031$), but only the 150 mg dose met the prespecified multiplicity-adjusted threshold for statistical significance. An additional analysis of the CANTOS trial demonstrated

Key advances

- The reduction of cardiovascular risk with a sodium–glucose cotransporter 2 inhibitor has been confirmed as a class effect[2]
- Glycaemic control is improved with a dual-acting angiotensin receptor–neprilysin inhibitor that also reduces the risk of heart failure and all-cause mortality[5]
- In a seminal finding, 2017 saw the first report of an anti-inflammatory agent, a monoclonal antibody targeting IL-1β, that reduces cardiovascular risk[6]
- An α-glucosidase inhibitor has been shown to reduce the rate of new-onset diabetes mellitus in Chinese people with coronary heart disease and impaired glucose tolerance

that canakinumab was associated with a higher incidence of fatal infection than the placebo; however, no difference in all-cause mortality was observed (HR (all doses) 0.94, 95% CI 0.83–1.06, $P=0.31$), possibly because this agent might also reduce the risk of some malignancies[7]. This first report of an anti-inflammatory agent reducing cardiovascular risk is a seminal finding that will stimulate a reinvigorated search for more effective anti-inflammatory interventions for atherothrombosis to help further reduce the residual cardiovascular risk seen in people with diabetes mellitus.

Epidemiological studies show that elevated post-challenge glucose levels are closely associated with an increased risk of type 2 diabetes mellitus and cardiovascular mortality. Acarbose, an α-glucosidase inhibitor, is licensed for the treatment of impaired glucose tolerance in China. In the acarbose cardiovascular evaluation (ACE) trial, 6,526 Chinese patients with known coronary heart disease and impaired glucose tolerance were randomly assigned to receive double-blind acarbose (50 mg three times daily) or a placebo, in addition to fully optimized cardiovascular risk factor management[8]. In 2017, after a median 5.0 year follow-up, the investigators reported that there was no reduction in the risk of major adverse cardiovascular events (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina or hospitalization for heart failure) in the acarbose group, but that the risk of diabetes mellitus was reduced by 18% relative to the placebo group, with a number needed to treat of 41 for 5 years to prevent one case of diabetes mellitus. In the longer term, it could be that acarbose might reduce cardiovascular risk indirectly, secondary to delaying the onset of diabetes mellitus in people with coronary heart disease and impaired glucose tolerance. Such an effect was observed in the Da Qing study, in which the delay in diabetes mellitus induced by a 6 year lifestyle intervention programme in Chinese people was subsequently associated with an 11.9% reduction in the incidence of cardiovascular death and a 28.1% reduction in all-cause mortality[9]. Given that ~500 million people in China currently have prediabetes[10], there are potentially major social, health and economic benefits to be gained by delaying the onset of diabetes mellitus in these individuals.

The key findings of 2017 include the coming of age of dual-purpose agents that not only lower blood levels of glucose but are also cardioprotective, as confirmed for the sodium–glucose cotransporter 2 inhibitor class and evidenced by sacubitril/valsartan. The first report of an anti-inflammatory agent that can reduce cardiovascular risk has been long awaited and might herald a paradigm shift in further improving cardiovascular outcomes for people with diabetes mellitus. Finally, using an α-glucosidase inhibitor to reduce the risk of diabetes mellitus in Chinese people with coronary heart disease and impaired glucose tolerance could help lessen a major health care challenge in China.

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NUTRACEUTICALS IN 2017

Nutraceuticals in endocrine disorders

Amanda J. Berberich and Robert A. Hegele

Nutraceuticals are gaining legitimacy and their potential clinical role is expanding. Data from 2017 provides evidence for their possible use in type 2 diabetes mellitus, the metabolic syndrome, obesity, dyslipidaemia and osteoporosis. Ongoing high-quality research in this area might justify future selective implementation of nutraceuticals into general health practice.

Nutraceuticals include a wide range of pharmaceutical-grade products that are subject to regulation as dietary supplements or food additives. They are purported to offer a multitude of health benefits, and companies can sell them directly to the public without going through the regulatory approval process that pharmaceuticals must go through. Nutraceuticals, which are often derived from food sources, are marketed with the general premise that they provide benefits beyond their basic nutritional value.

Historically, the legitimacy of the field has been compromised owing to a lack of high-quality scientific studies to support the, often exaggerated, health-benefit claims that are associated with commercialization and marketing of nutraceutical products. In many countries, relatively lax regulatory standards apply to nutraceuticals compared with traditional pharmaceutical agents, and unscrupulous manufacturers sometimes exploit this. The way in which some nutraceutical manufacturers

have exploited this has led to stigmatization of the field, which at times has overshadowed the potential for true benefit from a selected subgroup of these products.

While most nutraceuticals have an unsubstantiated role in evidence-based medical care, some are increasingly being subjected to rigorous scientific evaluation, and some of these even seem to show evidence of benefit for the treatment or prevention of disease. The studies discussed here, and others that are currently ongoing, might help transform the overall clinical landscape for these products in the treatment of endocrine conditions including lipid disorders, obesity, osteoporosis, hypothyroidism and type 2 diabetes mellitus.

For instance, the potential role of nutraceuticals in the management of dyslipidaemia has been of interest to researchers in the nutraceutical field. Lipid disorders are common, and the benefits of lowering low-density lipoprotein (LDL) cholesterol are well established[1]. Meeting ideal LDL cholesterol targets, however, can be difficult in some high-risk patients, especially in those who have an intolerance to statins. The 2017 ADHERENCE randomized clinical trial (RCT) included 100 high-risk patients who were currently on a tolerated statin but had levels of LDL cholesterol above guideline targets[2]. Participants were randomized to receive either low-dose statin with placebo or low-dose statin with Armolipid Plus (MEDA) formulation for 3 months. The Armolipid Plus formulation contains red yeast rice, policosanol, berberine, folic acid, coenzyme Q10 and astaxanthin[2]. Compared with the patients in the placebo group, the patients in the Armolipid Plus group had significantly reduced levels of total and LDL cholesterol, with 70% of participants meeting guideline targets, whereas none of the placebo group reached their target by the end of the trial[2]. The investigators observed no difference in rate of myalgia, which is the main statin-associated side effect that decreases compliance with treatment[2]. The Armolipid Plus nutraceutical formulation might represent a potential add-on therapy for patients who are intolerant of high-dose statin before considering newer agents, such as injectable inhibitors of PCSK9, which, while clinically effective, are costly[3].

The metabolic syndrome, and its related comorbidities, including polycystic ovary syndrome (PCOS), is another area of particular interest in nutraceutical research. One recent study specifically looked at the metabolic effects of soy isoflavones in PCOS[4]. This double-blind RCT included 70 women with PCOS who received either soy isoflavones or placebo for 12 weeks[4]. The investigators reported a significant improvement in markers of insulin resistance, including insulin resistance as defined by HOMA, insulin levels and the quantitative insulin sensitivity check index (QUICKI), as well as reductions in triglycerides and free androgen index[4]. In this RCT, the metabolic effects of PCOS were ameliorated somewhat, in some patients, with the use of soy isoflavones, which supports data collected from epidemiological and nutritional intervention studies and justifies further investigation into their use in women with PCOS.

Isoflavone aglycones used in conjunction with probiotics have also been shown to improve bone status in postmenopausal women with osteopaenia[5]. Oestrogen-based hormone replacement therapy can help attenuate bone loss, but data suggest it can also increase risk of breast cancer and vascular thrombotic events[6]. Isoflavones are thought to have mild oestrogenic effects that act via some of the same pathways as oestrogen-based hormone replacement therapy[5]. In a 12-month placebo controlled RCT, 78 postmenopausal women were treated with supplemental calcium, magnesium and calcitriol with either a masked placebo (control group) or red clover extract containing isoflavone aglycone and probiotics (treatment group)[5]. Women in the treatment group had significantly attenuated BMD loss compared with women in the control group[5]. The treatment group also showed an improvement in bone turnover markers, in their oestrogen metabolite profile and the levels of stimulated equol, which is an intestinal bacteria-derived nonsteroidal oestrogen receptor activator, compared with controls[5]. The trial was of insufficient duration to determine the safety of treatment with red clover extract containing isoflavone aglycone and probiotics with respect to breast cancer and fracture benefit. The trial does, however, provide evidence that the isoflavone and probiotic formula has measurable endogenous oestrogen-like effects that might translate into an effect on bone density.

Studies of prebiotics, probiotics and synbiotics have also shown promising results with regard to the treatment and prevention of disease. For instance, a recent study showed that a specific prebiotic reduces body fat in children who are overweight or obese[7]. In a double-blind, single-centre RCT, 42 children, aged 7–12 years, who were overweight or obese were randomly assigned to 16 weeks of treatment with oligofructose-enriched inulin (a prebiotic) or placebo[7]. Children who consumed the prebiotic showed a decrease in body fat and truncal fat, along with reductions in IL-6 and triglycerides, compared with children who consumed placebo[7]. In addition, a polymerase chain reaction and 16S ribosomal RNA faecal analysis revealed that the intestinal microbiome of the children treated with the prebiotic had been favourably altered, with significant increases in *Bifidobacterium* species — the same favourable effects were not observed in children in the control group[7]. This study provides support for the use of such prebiotics to aid with weight loss and to improve metabolic parameters in children who are overweight, an area of particular clinical and societal concern.

Key advances

- Nutraceuticals are migrating from the realm of pseudoscience into the arena of true science with an ever-increasing number of ongoing clinical trials and even evidence for benefit for some agents[2,4,5,7,9]
- Long-standing popular beliefs regarding the health effects of nutraceuticals are being tested and results are sometimes unexpected; some controlled studies failed to show benefit and some even demonstrated harm[10]. This highlights the importance of applying the high standards of clinical research to all potential therapeutic agents
- In selected common or under-treated conditions, where traditional pharmaceutical options are not available, undesirable or insufficient, nutraceuticals might be an effective therapeutic choice[2], and incorporating these agents into guidelines and treatment recommendations could be considered
- As each nutraceutical agent has different properties, mechanisms of action and intended effects[2,4,5,7,9,10], the evidence must be weighed appropriately for each individual agent prior to recommendation for use in certain clinical situations

Evidence for benefits of vitamin and mineral supplementation has been inconsistent over the past several decades[8]. A recent meta-analysis suggests that supplemental magnesium might help achieve blood pressure control in patients with prediabetes or other noncommunicable chronic diseases[9]. This meta-analysis included 11 RCTs, totalling 543 participants who were taking supplemental magnesium and had prediabetes, insulin resistance or a noncommunicable chronic disease, including coronary artery disease. The participants were followed up for between 1 month and 6 months, and their blood pressure was determined at the start and end of the study period[9]. In the pooled analysis, magnesium supplementation resulted in modest but significant reductions in both systolic and diastolic blood pressure[9]. These findings suggest that magnesium supplementation might be beneficial as adjunctive therapy for hypertension in a similar population, without any notable safety concerns.

Although the above results are promising and might justify further study of the respective nutraceuticals, an important negative study of resveratrol, a popular and widely available supplement found in red wine and grapes, is worth mentioning. This single-centre placebo-controlled RCT of 66 men examined the effect of resveratrol on parameters of the metabolic syndrome over 16 weeks[10]. The study found no difference for markers of inflammation, glucose homeostasis, blood pressure or hepatic lipid content[10]. Conversely, total cholesterol, LDL cholesterol and fructosamine levels were significantly increased in participants in the resveratrol treatment group[10]. These results highlight the importance of investigating the effects of supplements in a controlled setting to definitively evaluate both efficacy and unexpected negative consequences.

It is critical to weigh the evidence for each compound individually, just as for any drug, in order to avoid advising the use of a costly product that has no evidence for benefit, or even worse, a product that might actually cause unintentional harm. On the other hand, it is also important not to automatically dismiss nutraceutical products as having no role in modern evidence-based medicine. The studies discussed here are of reasonably good quality and some seemed to show short-term treatment benefits. Given the wide range of available products, their growing public popularity and the number of ongoing clinical trials, the field of nutraceutical medicine is likely to expand further, with potentially increased scientific legitimacy. However, it is essential that nutraceuticals and any evaluative trials are given the same due diligence that is provided to any other investigational product.

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GROWTH AND GROWTH DISORDERS IN 2017

Genetic and epigenetic regulation of childhood growth

Ola Nilsson

Studies of rare growth disorders taken together with large-scale genetic studies of adult height variability have uncovered a large genetic network regulating childhood growth. Advances in technology and experimental model systems will help decipher the molecular mechanisms of this complex network and lead to novel treatment approaches for growth disorders.

Linear growth is the result of chondrogenesis at the growth plates of long bones and vertebrae. Consequently, all forms of short stature are due to decreased chondrogenesis, whereas tall stature is due to increased growth plate chondrogenesis[1]. Insights from studying monogenic growth disorders as well as large-scale meta-analyses of genome-wide association studies (GWAS) of adult height indicate that growth is regulated by a large network of systemic and local factors that are important for growth plate chondrogenesis[1]. Interestingly, GWAS of normal variation of adult height have identified many of the same genes that are associated with monogenic growth disorders, which indicates that many genes involved in growth contain allelic series associated with a wide range of phenotypes ranging from severe growth disorders (such as skeletal dysplasias and primordial dwarfism syndromes) to common variants with mild effects contributing to variation within the normal range[2] (FIG. 1).

A new large study (combined sample size of 711,428 individuals) by the GIANT consortium identified 83 height-associated coding variants that are less common (minor allele frequencies in the 0.1–5% range) but have larger effects (up to 2 cm) than the more common variants identified by previous GWAS (mostly <5 mm)[3,4].

The study identified several rare variants in genes or pathways that were previously identified in a GWAS of human height and shown to be important for growth in monogenic growth disorders; for example, *IHH*, *PTH1R* and *NSD1*. This study thus confirms and extends the understanding of the growth-related allelic series ranging from monogenic disorders (such as skeletal dysplasias) with extreme short stature to common variants with small effects that contribute to the normal variation in adult height, with some genes associated with growth having variants that cause tall stature, for example, variants in *FGFR3* and *NPR2* (REF. 1) (FIG. 1).

Recent genome and/or exome sequencing studies of individuals with monogenic tall stature have identified mutations in several genes involved in creating and maintaining DNA and histone modifications (epigenetic genes) as novel causes of syndromic tall stature. In a study of 710 individuals with a combination of intellectual disability, and overgrowth and/or macrocephaly, Tatton-Brown *et al.*[5] identified the genetic cause in 50% (353 of 710) of the individuals and 44% (311 of 710) of the mutations were located in one of six genes involved in epigenetic regulation of gene expression, that is, *NSD1*, *EZH2*, *DNMT3A*, *CHD8*, *HIST1H1E* and *EED*. Mutations in *NSD1* that caused Sotos syndrome were found in 34% of the participants (240 of 710) and thus accounted for as much as two-thirds of all identified mutations[5].

The mechanism by which mutations in epigenetic genes causes statural overgrowth is not clear but is presumably mediated by altered gene expression in growth plate cartilage. For example, Weaver syndrome is due to mutations in *EZH2* and was the second most common finding (4.8%) in the cohort of the childhood overgrowth collaboration[5]. EZH2 is part of the polycomb repressive complex 2 (PRC2), which is comprised of four subunits (EED, SUZ12, RBBP4 and EZH1 or EZH2) and is responsible for trimethylation of histone 3 at lysine 27 (H3K27). As a first step to characterize the mechanism by which these mutations cause overgrowth, Imagawa *et al.* studied H3K27 trimethylation in patients with overgrowth and intellectual disability due to deletion or mutation of *EZH2*, *EED* or *SUZ12* and found evidence that mutations in *EED* and *SUZ12*, like the loss-of-function *EZH2* mutations in Weaver syndrome, result in decreased H3K27 trimethylation by the PRC2 complex[6,7]. In addition, previous studies on genetic targeting of *EED* or *EZH1* and *EZH2* in mice have implicated several signalling pathways that are important for proliferation and differentiation in growth plate chondrocytes, including the Wnt, transforming growth factor-β (TGFβ) and insulin-like growth factor (IGF) pathways as well as cyclin-dependent kinase inhibitors Ink4a/4b[8,9].

Key advances

- Rare variants in genes encoding proteins that function in the growth plate provide moderate-to-large effects on normal variation of adult height[4]
- Mutations in genes important for epigenetic regulation are major causes of overgrowth and intellectual disability syndromes[5]
- Weaver syndrome is due to heterozygous loss-of-function mutations in components of the polycomb repressive complex 2, including *EZH2*, *EED* and *SUZ12*[6]
- Identification of gene variants in the gene encoding the retinoic acid catabolizing enzyme *CYP26C1*, that act as genetic modifiers of the SHOX deficiency phenotype, demonstrate a successful approach to a digenic condition[10]

The dramatic improvement and reduced cost of DNA sequencing have accelerated the discovery of genetic aetiologies of monogenic conditions. By contrast, identifying the genetic causes of polygenic conditions is more difficult. Heterozygous loss-of-function mutation or deletion of *SHOX* causes short stature ranging from disproportionate (mesomelic) short stature with or without Madelung deformity of the wrist and forearm bones, associated with Leri–Weill dyschondrosteosis, to mild, isolated short stature (FIG. 1). Montalbano *et al.*[10] studied a three-generation family with a missense mutation (p.Val161Ala) of a highly conserved amino acid in the DNA-binding domain of SHOX and found that the variant had a strong effect on the transcriptional activity of the mutated SHOX protein. However, only individuals who also carried a variant in *CYP26C1* that resulted in decreased metabolism of retinoic acid displayed the full phenotype[10]. The suggested mechanism, supported by studies in human chondrocytes and knockdown of SHOX and/or CYP26C1 in zebrafish, includes decreased CYP26C1 activity that leads to increased levels of retinoic acid, which in turn reduces SHOX expression and thereby enhances the effect of the *SHOX* variant in the individuals carrying both the

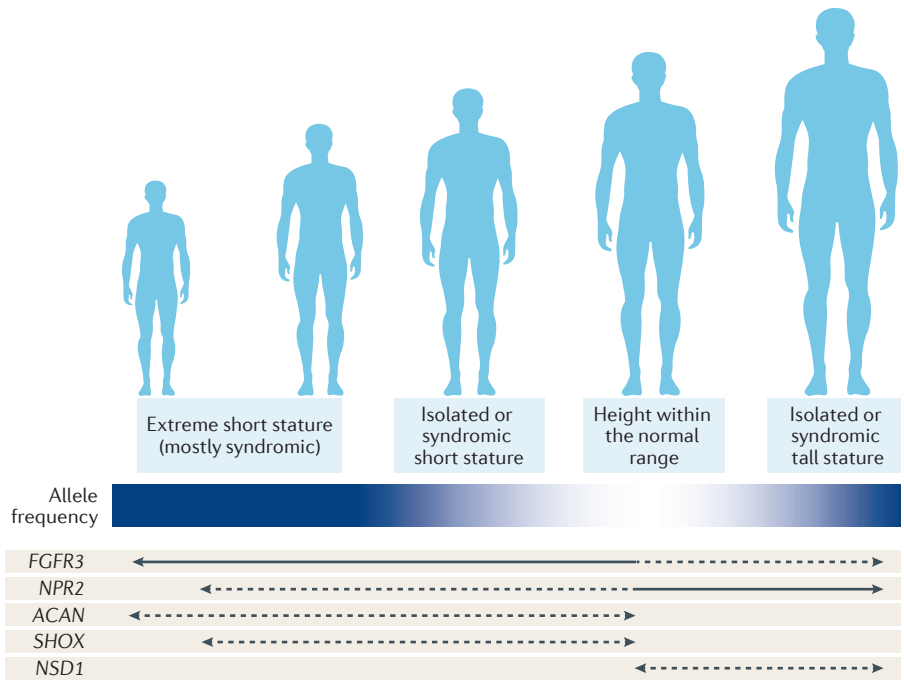

Figure 1 | **Allelic series in growth genes.** Variants in the same gene can have a wide spectrum of effects ranging from extreme short stature in monogenic growth disorders to common variants with small effects that contribute to the variation in adult height within the normal range. In some genes, including *FGFR3* and *NPR2*, mutations can, depending on the effect on the protein (loss of function or gain of function), cause either short or tall stature. Solid arrow, gain-of-function; dashed arrow, loss-of-function; dark blue, extremely rare; white, common.

SHOX and *CYP26C1* variants[10]. This study is one of only a handful of studies implicating genetic modifiers in monogenic conditions, which points towards the next big challenge in research of genetic conditions — to identify the underlying genetic causes of digenic and oligogenic conditions.

In summary, these four studies[4–6,10], published in the past 12 months, provide new insight into the genetic regulation of growth and the pathogenic mechanisms of growth disorders. Identification of rare genetic variants that have larger effect sizes with magnitudes between those identified by GWAS and the variants causing monogenic growth disorders adds a missing piece in the complex genetic puzzle of growth regulation and reinforces the concept that genetic variants in the same genes associated with growth can cause a wide spectrum of phenotypes (FIG. 1). Furthermore, studies of monogenic tall stature demonstrate that mutations in epigenetic regulators are common causes for syndromes of overgrowth, macrocephaly and mental disability. Finally, identification of variants in the gene that encodes the retinoic acid metabolizing enzyme CYP26C1 as a genetic modifier of SHOX deficiency represents a first step towards the understanding of oligogenic growth disorders. Most importantly, the continuing progress in the field provided by these and other studies published during the past year provides novel therapeutic targets that could be exploited to develop new treatment strategies for growth and other skeletal disorders.

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IMMUNOMETABOLISM IN 2017

Metabolism and the inflammasome in health and ageing

Thomas Mandrup-Poulsen

Extracellular danger-associated molecular patterns signal to NOD-like receptors, but the exact signalling pathways remain unclear. The inflammasomes, a subgroup of these receptors, translate danger signals into inflammatory responses by maturing IL-1 and IL-18. In 2017, researchers reported novel functions of the mutual interaction between metabolism and the inflammasomes in health and disease.

Inflammation is a physiological response that helps restore homeostasis following infectious, immune-mediated and physicochemical tissue injury. The protective properties of inflammation can be altered by insults that have a high intensity and/or long duration, or if the magnitude of counter-regulatory measures are perturbed. A damaging inflammatory response can result in collateral tissue dysfunction, destruction, remodelling and fibrosis. The apprehension that the metabolism of immune cells is critical for their activation, proliferation, function and decay[1], and that immune cells and their many mediators affect metabolic processes in most non-immune cells[2], prompted the emergence of the term immunometabolism.

Immune cells sense metabolites as danger signals via the inflammasome, a multiprotein complex from the NOD-like family of danger-associated molecular pattern receptors, and thereby translates a systemic dysmetabolic environment into humoral signals that regulate the responses of adipocytes, skeletal muscle, hepatocytes and the endocrine and neuroendocrine systems to metabolic stress[3,4]. The NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome relays signals from glycolysis and mitochondrial metabolism to the innate immune system, which activates caspase 1 to cleave its pro-inflammatory substrates, pro-IL-1β and pro-IL-18, into active IL-1 and IL-18, respectively[5]. The molecular sensing mechanism of the inflammasome activating caspase 1 is still unclear.

Type 2 diabetes mellitus (T2DM) is suggested to be of auto-inflammatory nature, as metabolites activate the inflammasome. IL-1 inhibits pancreatic β-cell function and causes its apoptosis, and antagonism of IL-1 improves β-cell function in patients with T2DM[6]; however, the evolutionary advantage of nutrient sensing by the inflammasome is unclear. One study[7] demonstrated that the transient low-grade inflammatory state induced shortly after a meal in healthy individuals establishes a feedforward loop with dual homeostatic purposes. Postprandial hyperglycaemia activates the omental fat macrophage inflammasome via glucose transporter 1-mediated glucose uptake, glucose oxidation and reactive oxygen species formation to generate mature IL-1, which then potentiates glucose-stimulated insulin secretion[7]. This insulin secretion augments

macrophage glucose uptake and promotes further IL-1 production, whereby insulin and IL-1 in synergy boost glucose disposal. This feedforward loop ensures that sufficient nutrient metabolism and storage occurs in insulin-sensitive tissues and that there is energy for innate immune cells to deal with potential infections from microorganisms in food.

The frequency of chronic morbidity associated with an unhealthy lifestyle, systemic low-grade inflammation and inflammasome activation increases with age (which is known as 'inflammageing'). Ageing is also associated with reduced lipolysis and substrate mobilization, which explains why elderly individuals have ectopic visceral fat deposition, impaired exercise capacity and cold intolerance. The underlying mechanisms of impaired lipolysis in ageing have been unknown and cannot be explained by impaired signalling of catecholamines, the major driver of lipolysis.

Macrophages can infiltrate ectopic fat, and one study[8] demonstrated that adipose tissue macrophages from aged mice inhibit catecholamine-stimulated lipolysis. Transcriptomic analyses of adipose tissue macrophages from aged mice demonstrated an unexpected upregulation of catecholamine-degrading enzymes, including monoamine oxidase A (MAOA). This upregulation of catecholamine-degrading enzymes was dependent on inflammasome activity inducing growth/differentiation factor 3 (GDF3), a transcription factor for MAOA expression. A direct effect of IL-1 or IL-18 on the expression of GDF3 was not investigated. A feedforward loop is established as GDF3 also drives inflammasome transcription. In GDF3-null mice, increased lipolysis and reduced inflammasome-induced expression of MAOA was observed. Finally, adipose tissue macrophages were in close physical association with sympathetic nerve endings, suggesting that these macrophages are involved in degradation of released catecholamines. These findings have translational perspectives as the MAOA inhibitor clorgyline restored lipolysis in adipose tissue *in vitro* and *in vivo*.

RNA sequencing analysis of mouse adipose tissue macrophages[8] revealed an enrichment of genes implicated in cell senescence that are regulated by the inflammasome. Another group reported[9] a similar observation using a different approach. The authors used the Stanford–Ellison prospective population cohort and, using genomic profiling, demonstrated that the expression of inflammasome gene modules correlated positively with human ageing. The investigators also selected for extreme phenotypes by stratifying based on inflammasome gene modules that are linked to stable high or low circulating levels of IL-1, based on their expression level (high or low, respectively). The team reported correlations with longevity and the cardiovascular risk markers hypertension and carotid arterial stiffness. A question that arises is how does ageing activate the inflammasome? A metabolomic analysis of these extreme phenotypes revealed dysfunction of nucleotide metabolism in participants with the inflammatory genotype that coexisted with increased chronic oxidative stress markers. Combinations of nucleotide metabolites stimulated inflammasome expression and activity *in vitro* and elicited hypertension and inflammatory signatures *in vivo*.

As caffeine is an antagonist of the nucleotide metabolite adenosine, the authors asked study participants to detail their caffeine consumption and measured caffeine and its metabolites in the plasma of participants in the extreme phenotype group. Participants in the 'low' expression inflammasome module group had high intake and circulating levels of caffeine, the same concentrations that inhibited inflammasome activation *in vitro*. This study demonstrates an association between inflammasome activity and cardiovascular risk. What remains unclear from these studies, however, is whether the inhibition of the downstream processes caused by the activation of the inflammasome prevents cardiovascular disease.

The inflammatory hypothesis of atherosclerosis, proposed in the 1980s, is based on circumstantial evidence. In one study[10], >10,000 patients with previous myocardial infarction and systemic low-grade inflammation were randomly assigned to receive either

Key advances

- The inflammasome contributes to glucose homeostasis[7]
- The inflammasome drives catecholamine degradation, which blunts lipolysis, and in turn predisposes elderly individuals to visceral adiposity and reduced substrate mobilization[8]
- Nucleotide metabolites activate the inflammasome, which contributes to hypertension, platelet activation and arterial stiffness in elderly individuals[9]
- Assessment of inflammasome activity can predict cardiovascular risk[9]
- Neutralising the inflammasome product IL-1 reduces cardiovascular morbidity and mortality[10]

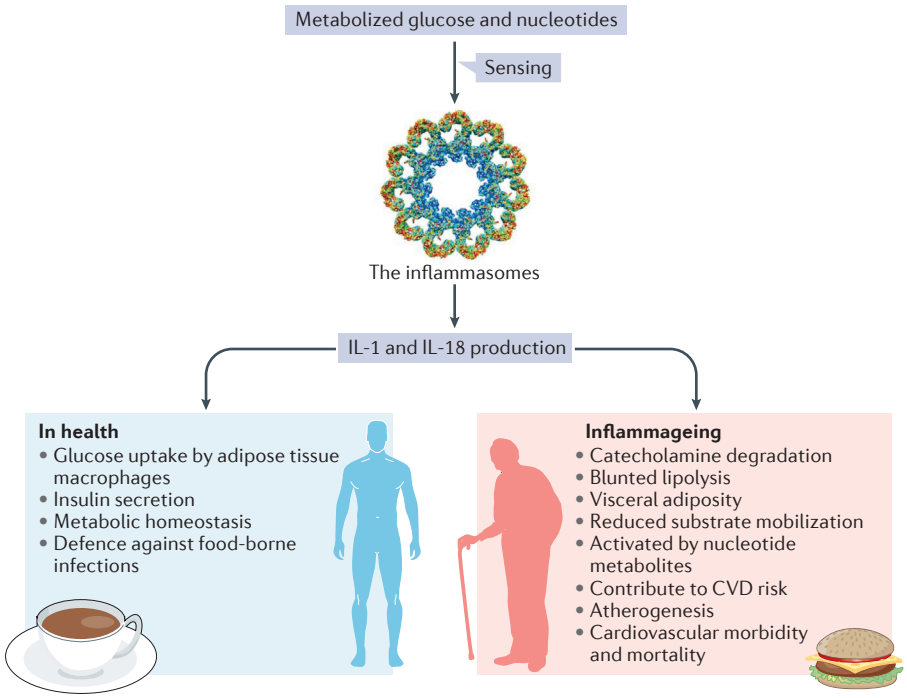

Figure 1 | **The inflammasome and metabolism in homeostasis and inflammageing.** Inflammasomes sense and are activated by the metabolism of substrates, such as glucose and nucleotides. The activation of inflammasomes leads to the production of pro-inflammatory cytokines IL-1 and IL-18, which contribute to the homeostatic response following the ingestion of a meal and to inflammatory diseases that are associated with old age, such as cardiovascular diseases (CVD). Caffeine can counteract the problems associated with the activation of inflammasomes.

subcutaneous injections of three different doses of IL-1β antibody or a placebo every 3 months and were followed up for a median of 3.7 years.

The authors reported that the IL-1β antibody dose-dependently reduced inflammatory markers by 25–43%. Notably, the treatment had no effect on lipids. The primary composite end point (nonfatal myocardial infarction, nonfatal stroke or cardiovascular death) was reduced by 15–17%. The reporting of prespecified diabetes mellitus end points is expected in March 2018. Of concern, the authors reported an increased risk of fatal infection in frail elderly patients. Whether this fatality could be prevented by the use of more readily reversible anti-IL-1 therapies, such as the IL-1 receptor antagonist, remains to be tested.

The studies selected here demonstrate that transient, balanced inflammasome activation serves homeostatic purposes, whereas persistent, unopposed inflammasome activity is a central component of diseases in inflammageing (FIG. 1). Fortunately, both pharmacological and dietary interventions may prevent the adverse effects of chronic, unbalanced inflammasome activation reviewed here. To cite Mallar Bhattacharya's commentary in *Science Translational Medicine* on Furman and colleagues' study[9]: maybe a coffee a day will keep the inflammasome away!

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Competing interests statement
The author declares no competing interests.

THE ARTIFICIAL PANCREAS IN 2017

The year of transition from research to clinical practice

Boris Kovatchev

The artificial pancreas — the automated closed-loop control of diabetes mellitus — made its first outpatient strides in 2011. In 2017, the results of long-term clinical trials on the artificial pancreas were published, the first hybrid commercial artificial pancreas system was approved and the artificial pancreas was tested under increasingly demanding conditions. Thus, artificial pancreas technology is here to stay.

People with type 1 diabetes mellitus (T1DM) face a lifelong optimization problem to maintain strict glycaemic control without increasing their risk of hypoglycaemia. Over the past 40 years, the engineering response to this clinical demand has progressed through continuous subcutaneous insulin infusion (CSII) using insulin pump, real-time continuous glucose monitoring (CGM) and mathematical algorithms linking CGM and CSII to enable automated closed-loop control known as the artificial pancreas[1]. During 2008–2010, promising inpatient results reported by several groups pointed out the superiority of closed-loop control over CSII therapy in terms of increased time within a recommended blood glucose target range of 3.9 mmol/l to 10 mmol/l, reduced incidence of hypoglycaemia and better overnight control.

The first outpatient trials using a portable artificial pancreas system were conducted in October 2011 and were expanded thereafter into increasingly longer and larger studies within patients' natural environments[2,3]. In September 2016, the first safety pivotal trial of a commercial hybrid closed-loop system — the Medtronic MiniMed 670G — was reported[4] and the artificial pancreas began its transition from a research subject to clinical practice.

Based on a PubMed search of "artificial pancreas diabetes", in 2017, >80 scientific publications were related to the artificial pancreas. Here I highlight the studies from 2017 that support the transition of closed-loop control to routine clinical use by expanded analysis of long-term glycaemic outcomes[5], testing algorithms that offer adaptation to an individual patient[6,7] and assessing the performance of the artificial pancreas during physical activity[8], including under demanding winter-sport conditions[9].

Following a summary published in *JAMA*[4], Garg and colleagues[5] reported detailed glycaemic control outcomes in adolescents (ages 14–21 years) and adults who were using hybrid closed-loop — an artificial pancreas system that automates insulin basal rate using CGM signal and a control algorithm, but does not administer automated postprandial insulin corrections or premeal boluses. During hybrid closed-loop use, there were no severe hypoglycaemic or diabetic ketoacidosis events and average glycaemic control improved from baseline to the end of the study, as indicated by HbA_{1c} reduction from 7.7% to 7.1% in adolescents and from 7.3% to 6.8% in adults. Concurrently, CGM-based metrics, such as the time within the target range of 3.9 mmol/l to 10 mmol/l, improved as well. Thus, while this study did not have a randomized design and a control group, the substantial cohort size ($n = 124$ patients with T1DM, including 30 adolescents) and the 3-month duration of the clinical protocol allowed the investigators to test the safety of in-home use of the hybrid closed loop. This trial resulted in the subsequent regulatory approval of the system by the FDA, which opened the artificial pancreas to routine clinical use.

In a randomized crossover study[6], the control algorithm was first initialized by patient-specific parameters, such as body weight, total daily insulin and basal insulin pattern, but it further adjusted itself during closed-loop operation. The hardware used in this study was complicated and included a number of components, including a CGM receiver, a purpose-made translator converting the CGM signal to Bluetooth and a smart phone running the algorithm, but the control algorithm was conceptually advanced, representing a step towards an automated personalized artificial

pancreas. In this study, the coordinators randomized 29 adults who had well-controlled T1DM (HbA_{1c} <7.5%) and showed that during the two 4-week periods of closed-loop control, the CGM-based time within the target range of 3.9 mmol/l to 10 mmol/l increased by 10.5%, compared with the control periods where sensor-augmented pump therapy was used. The crossover study design was particularly powerful in showing that the artificial pancreas system was able to achieve notable glycaemic improvement, even in people who had good glycaemic control at baseline.

As metabolic parameters, such as insulin sensitivity, rate of subcutaneous insulin transport or rate of glucose absorption from meals, vary between people and within each person over time, it is commonly accepted that closed-loop algorithms should adapt to each individual. Dassau and colleagues brought us a step closer to personalized artificial pancreas systems with a 12-week multicentre trial of 24/7 adaptive closed-loop control[7]. In this trial, the insulin requirements of each participant (basal rate settings and carbohydrate ratio) were adapted every week using an adaptation algorithm. Consistent with the pilot nature of this study, the adaptation of these critical parameters had to be confirmed by a physician before the artificial pancreas was implemented. Twenty-nine patients completed the trial and their HbA_{1c} improved from an excellent baseline average of 7% to 6.7% at the end of the study. This change in HbA_{1c} was accompanied by concurrent improvement in the frequency of CGM readings below 3.9 mmol/l from 5.0% at baseline to 1.9% at the end of the study. Thus, despite lacking a control group, this study demonstrated the feasibility of an adaptive closed loop, which, in this study, improved simultaneously the two key parameters of diabetes mellitus control: average glycaemia and exposure to hypoglycaemia.

To become viable for everyday use, artificial pancreas systems must be able to respond to a variety of metabolic challenges, including meals containing fast-acting or slow-acting carbohydrates, and metabolic perturbations triggered by physical activity of varied intensity and duration. Although some artificial pancreas systems deliver automated boluses to correct hyperglycaemia[9], and experiments with fully automated controllers are ongoing, most contemporary solutions still rely on counting meal carbohydrates and manually delivered premeal boluses, a process that helps to minimize postprandial glycaemic excursions. To evaluate the performance of an artificial pancreas system during and after physical activity, Dovc and colleagues[8] conducted an inpatient randomized controlled crossover trial, in which children and adolescents with T1DM participated in moderate-intensity and high-intensity exercise sessions. Compared with open-loop control, closed-loop control increased the CGM-based time within the target range of 3.9 mmol/l to 10 mmol/l from 68.7% to 84.1%, while the percentage readings below 3.9 mmol/l were low throughout the trial and remained unchanged. The study concluded that exercise can be safely handled by a closed-loop system during 22-hour sessions in a hospital setting.

Key advances

- Following approval by the FDA of the first hybrid closed-loop system, the artificial pancreas began its transition from a topic of research and development to the clinical practice[4,5]
- Adaptive closed-loop control algorithms were tested that adjust key parameters of insulin delivery to the individual metabolic characteristics of each person and adapt them over time[6,7]
- Artificial pancreas systems were tested during and after intense physical activity[8], including under the demanding winter-sport conditions of an artificial pancreas ski camp (cold, altitude and lengthy exercise)[9]
- The NIH supported four large-scale studies, with the goal to establish closed-loop control as a viable, clinically accepted treatment for type 1 diabetes mellitus[10]

In the first artificial pancreas ski study, Breton and colleagues tested the response of closed-loop control to the rather extreme real-life challenges of lengthy outdoor winter-sport activities[9]. This randomized controlled trial enrolled 32 adolescents in two ski camps with T1DM who skied 5 hours every day for 5 days. The study began at a relatively low altitude (1071 m) in Virginia and then continued with a second camp in Colorado (3914 m at the summit). Compared with remotely monitored sensor-augmented pump therapy, the artificial pancreas resulted in improved CGM-based time in the target range of 3.9 mmol/l to 10 mmol/l (71.3% versus 64.7%) and reduced rate of CGM readings below 3.9 mmol/l (1.8% versus 3.2%), with maximum effect late at night. It was therefore concluded that a contemporary artificial pancreas system can handle the metabolic perturbations triggered by demanding winter-sport conditions, including cold, altitude, lengthy exercise, excitement and possibly fear.

In conclusion, the five studies highlighted here represent key milestones in the quest for clinically viable artificial pancreas systems. Two types of design — embedded and mobile — have emerged as alternatives for artificial pancreas implementation (FIG. 1), and both are tested in large-scale ongoing trials supported by the NIH[10]. Thus, we can be optimistic that the closed-loop artificial pancreas will fulfil its promise of becoming the digital-age treatment for T1DM in the near future[3].

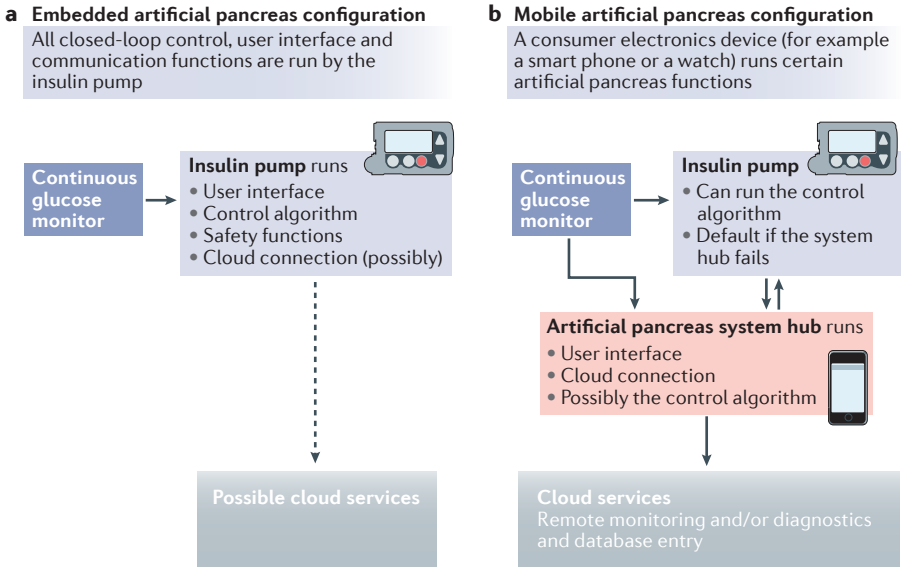

Figure 1 | **Embedded and mobile artificial pancreas system configurations.** Artificial pancreas system configurations include all-in-one systems (part **a**), in which all the functions of the artificial pancreas are perrformed by the insulin pump, and mobile solutions (part **b**) that use consumer electronics for certain functions, including to run the artificial pancreas algorithm. Both configurations are currently being tested for clinical use.

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GUT MICROBIOTA IN 2017

Contribution of gut microbiota–host cooperation to drug efficacy

Nathalie M. Delzenne and Laure B. Bindels

2017 has witnessed key advances in knowledge about the metabolic capacities of the gut microbiota, enabling the progression of our understanding of the principles driving xenobiotic–bacteria–host interplay. This research paves the way for the long road towards personalized medicine and nutrition, which could be based on gut microbial metabolism.

The gut microbiota encompasses all microorganisms present in the gut, including bacteria, viruses, phages, parasites and fungi. These gut microorganisms are able to modulate host metabolism and immunity, mainly through the production of metabolites and the release of bioactive components, and thereby, influence health status and disease occurrence and/or progression[1]. The variety in microbial metabolites results from the wide metabolic potential buried in the microbial genome. Although gut bacteria currently represent the best characterized component of the gut microbiota, the gut bacterial metabolome is still considered a black box for the most part. With the exception of bile acids and short-chain fatty acids, most bacterial metabolites are poorly characterized from a structural point of view and their relevance and physiological roles remain unknown. However, as the human microbiota is highly individualized, we can speculate that gaining sufficient knowledge about how the microbial metabolism of xenobiotics and nutrients enables the release of bioactive compounds able to act on host cells will be an essential and unavoidable step towards successfully harnessing the therapeutic potential of the gut microbiota. Such knowledge will, for instance, foster the successful application of nutritional microbiota-based therapeutics or enable the optimization of the efficacy of microbiota-interacting drugs in humans. Four studies published in 2017 paved the way in this direction by unravelling the nature and physiological role of previously unknown bacterial metabolites involved in the fine regulation of host metabolism, host proteases and drug response by gut bacteria (FIG. 1).

In August 2017, Cohen *et al.*[2] revealed that bacteria present in the human gut can produce small lipid metabolites able to specifically modulate the activity of several G protein-coupled receptors (GPCRs). In this elegant, powerful, yet easy-to-follow study, Cohen and his colleagues started by identifying several *N*-acyl synthase genes present within the human microbial genome using sequence data from the Human Microbiome Project (HMP). These genes were then synthesized, expressed in *Escherichia coli* and their lipid products structurally defined. Using high-throughput GPCR activity screening, they pinpointed several GPCRs whose activity was increased or inhibited by these lipid products, thereby revealing new pairs of microbial ligands and GPCRs. For instance, the authors convincingly showed that *N*-oleoyl serinol produced by bacteria such as *Gemella* spp. can activate GPR119 with a similar potency to the endogenous ligand oleoylethanolamide. GPR119 is an attractive therapeutic target for diabetes and obesity because this receptor affects glucose homeostasis, among others, through the release of glucagon-like peptide 1 (GLP1). Along these lines, colonization of antibiotic-treated mice with an *E. coli* strain engineered to express the *N*-acyl serinol synthase gene improved glucose tolerance and increased GLP1 secretion after glucose administration, compared with *E. coli* engineered to express an *N*-acyl synthase point mutant as a control. As pointed out by the authors themselves, even if these lipid products are actually produced by the human gut microbiota, the extent to which these microbial products contribute to host physiology remains undetermined so far. Nonetheless, this pioneer study clearly sheds light on previously ignored bacterial bioactive lipids and demonstrates the potential of microorganisms genetically engineered to produce small bioactive lipids as biotherapeutic tools.

Guo *et al.*[3] used a similar approach to that of Cohen and colleagues, combining bioinformatics mining and heterologous gene cluster expression with unbiased chemical proteomics experiments to reveal a new type of bacterial metabolite. This study focused on bacterial dipeptide aldehydes produced by nonribosomal peptide synthetase gene clusters found to a large extent in bacterial strains from the class Clostridia. The researchers showed that these dipeptide aldehydes are able to specifically inhibit a subset of human cathepsins, mainly cathepsins L. One interesting hypothesis by the authors concerns the role of this interspecies interaction. They proposed that dipeptide-aldehyde-mediated cathepsin inhibition could enable gut microorganisms to stably occupy phagolysosomes in gut epithelial or immune cells, thereby modulating the human intestinal immune system. In the Cohen *et al.* study[1], the precursors for such metabolites are likely glycerol and fatty acids, whereas for the Guo *et al.*[2] study,

Key advances

- Bacterial lipid products are able to modulate host GPCR activity: for instance, *N*-oleoyl serinol is a GPR119 agonist able to modulate GLP1 secretion and glucose homeostasis[2]
- Dipeptide aldehydes are widely produced by gut microorganisms and can selectively inhibit the activity of a subset of human cathepsins[3]
- Bacteria can increase the activity of 5-fluorouracil, a chemotherapeutic agent, by converting it into 5-fluorouridine monophosphate through the bacterial ribonucleotide metabolism pathway[5,6]

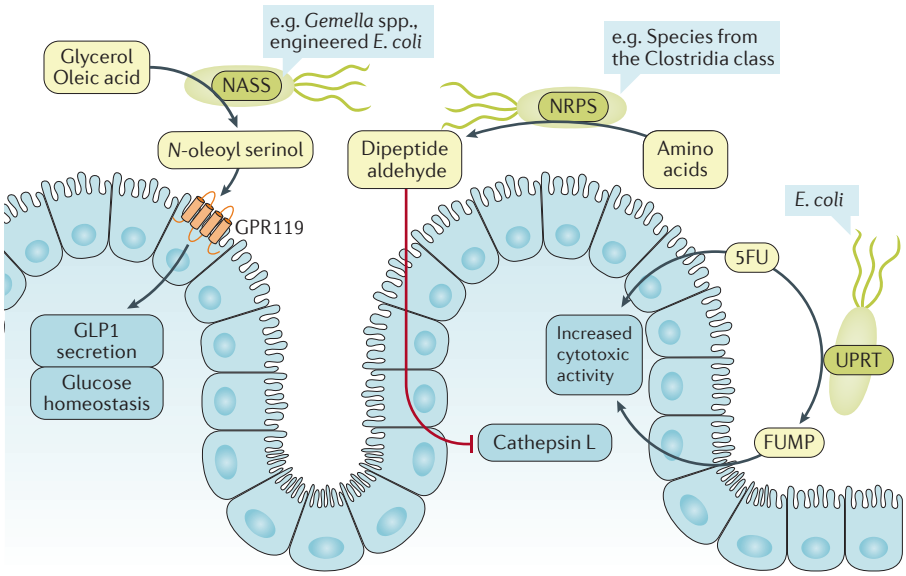

Figure 1 | **New metabolic pathways discovered in 2017 that underlie the xenobiotics–microbiota–host interplay.** *N*-oleoyl serinol can be produced by bacteria such as *Gemella* spp. through *N*-acyl serinol synthase (NASS) and can activate the GPR119 receptor, thereby modulating glucagon-like peptide 1 (GLP1) secretion and glucose homeostasis. Dipeptide aldehydes produced by bacterial strains from the class Clostridia, via a nonribosomal peptide synthetase (NRPS), can inhibit human cathepsins L and could contribute to host intestinal immune regulation. Bacteria such as *Escherichia coli* can increase the cytotoxic activity of the chemotherapeutic agent 5-fluorouracil (5-FU) by metabolizing it into 5-fluorouridine monophosphate (FUMP), a RNA damaging agent, through the UPRT enzyme (uracil phosphoribosyltransferase) encoded by the *upp* gene.

the precursors for dipeptide aldehydes are probably protein-derived amino acids. These findings suggest new bacterial pathways that enable the production of modulators of key host functions from nutrients.

Bacterial byproducts can also be released after a microbial interaction with xenobiotics (including drugs and environmental toxicants). Such drug–microorganism interactions can affect the efficacy and toxicity of the xenobiotic towards the host[4]. In 2017, Scott *et al.*[5] and Garcia-Gonzalez *et al.*[6] went one step further in detailing this complex interplay between the host, its microbiota and xenobiotics. Using an original holobiont model composed of the nematode *Caenorhabditis elegans* and a single bacterial strain, both studies showed that bacteria can metabolically complement *C. elegans* to modulate the effects of a chemotherapeutic prodrug agent, 5-fluorouracil. Specifically, using large collections of bacterial mutants, both teams consistently revealed that 5-fluorouracil can be transformed into 5-fluorouridine monophosphate, which will impair host RNA synthesis, by the bacterial ribonucleotide metabolism pathway.

Additional studies using human samples and mammalian species with complex microbiota receiving the drug in a method similar to that used in clinics (intravenous injection in the case of 5-fluorouracil) will be needed to answer the final key question: what is the contribution of the gut bacteria versus the host to the activation of this drug in humans? Nonetheless, if these findings are corroborated in the future by human data, they could be of tremendous help in the context of personalized medicine. Indeed, most chemotherapeutic agents have a narrow therapeutic index. Accurate prediction of the pharmacokinetics of these chemotherapeutic agents represents a hot topic of research because of its potential to both improve efficacy and reduce toxicity of such drugs, thereby reducing the risk of therapeutic failure. The work of Scott *et al.*[5] and Garcia-Gonzalez *et al.*[6] provides evidence that the bacterial ability to metabolize chemotherapeutics such as 5-fluorouracil might become one of the parameters, along with host genotype, able to predict the pharmacokinetics of drugs.

The human relevance of the research linking microbiota and xenobiotics can be unravelled through the use of publicly available metagenomic and metatranscriptomic data. Cohen *et al.*[1] and Guo *et al.*[2] were able to show that genes encoding the synthases that allow the production of metabolites of interest were heavily present in the human microbiota, and that these genes were transcribed to various extents. For instance, at the DNA level, Cohen *et al.*[2] showed that most gene families encoding for *N*-acyl synthases were found in >90% of the HMP stool samples ($n = 148$), whereas Guo *et al.*[3] reported that at least one of the 47 clusters coding for their nonribosomal peptide synthetase was present in 88% of these samples ($n = 149$). Clearly, these studies illustrate how we can collect the fruit of the community effort started a few years ago aiming to better characterize the human gut microbiome, with projects such as the MetaHIT[7] and the HMP[8], and stress now more than ever the importance of big data sets to GO-FAIR (the Global Open (GO)-FAIR Initiative aims to put in practice the FAIR principles, namely, that data should be findable, accessible, interoperable and reusable by both humans and machines[9]).

In conclusion, innovative approaches to studying the complex interactions between the gut microbiota and host revealed new metabolites resulting from bacterial transformation of xenobiotics and nutrients. The management of drug efficacy and/or toxicity might need to take into consideration the huge panel of metabolic pathways developed by commensal microorganisms. We now have more impetus to pay attention to the microbiota when evaluating the development of personalized medicine and nutrition.

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Competing interests statement
The authors declare no competing interests.

NAFLD IN 2017

Novel insights into mechanisms of disease progression

Reenam S. Khan and Philip N. Newsome

In 2017, there have been substantial advances in our understanding of the immunological and endocrine mechanisms of disease progression in NAFLD, paving the way for novel therapeutic strategies.

NAFLD encompasses a spectrum of conditions ranging from simple steatosis to NASH, which is characterized by hepatic inflammation and fibrosis and an increased risk of progression to liver cirrhosis and development of hepatocellular carcinoma (HCC). The prevalence of NAFLD varies worldwide, but has been estimated to be 25% globally[1]. NAFLD is closely associated with the metabolic syndrome, hence the incidence of NAFLD has been rising in parallel with the increasing incidence of diabetes and obesity, and might soon become the leading cause of liver transplantation in Europe and the USA[2].

Apart from limited evidence to suggest that there could be some improvement in histological features of NASH with thiazolidinediones and vitamin E in specific patient subgroups, there is currently no effective licensed pharmacotherapy for NAFLD. As simple steatosis is relatively benign, strategies that halt progression of steatosis to NASH have the potential to markedly reduce NAFLD-associated morbidity and mortality. Development of such therapies has thus far been hampered by an insufficient understanding of mechanisms that mediate this transition. Factors implicated include oxidative stress, mitochondrial dysfunction, lipotoxicity and immune cell activation (FIG. 1), and in a number of chronic liver diseases, it has been shown that hepatocyte death triggers inflammation as a mechanism for liver regeneration[3].

Work by Guo *et al.*[4] published in 2017 suggested the role of hepatic neuregulin 4 (NRG4) as an endocrine 'checkpoint' for the development of NASH. NRG4 is highly expressed by adipose tissue and binds to ERB3 and ERB4 receptor tyrosine kinases on hepatocytes, activating a signalling pathway that attenuates the hepatic lipogenic response and inhibits degradation of CASP8 and FADD-like apoptosis regulator (CFLAR, also known as c-FLIP). Of note, CFLAR protects against hepatocyte death, and this study demonstrated that patients with NASH had higher levels of hepatocyte death markers, lower levels of NRG4 and lower levels of CFLAR than healthy individuals. *Nrg4*$^{-/-}$ mice that were exposed to a diet to induce NASH developed accelerated liver injury, fibrosis and cell death compared with wild-type mice. Notably, this effect was reversed by adenovirus-mediated restoration of CFLAR levels in *Nrg4*$^{-/-}$ mice. Furthermore, mice with transgenic expression of NRG4 developed a milder phenotype of NASH on exposure to the relevant diet. Thus, manipulation of this NRG4–CFLAR pathway represents a novel potential therapeutic strategy in NAFLD. Previous evidence highlights an inverse correlation between serum NRG4 levels and the presence of subclinical cardiovascular disease in individuals who are obese, hence manipulating this pathway could also help to reduce cardiovascular disease risk[5].

Further work by Hart *et al.*[6] has developed our understanding of the immunological mechanisms of disease progression in NAFLD. Obesity is associated with the development of type 1 inflammatory responses in adipose tissue, yet Hart *et al.*[6] demonstrated that in NAFLD, a type 1 inflammatory response, induced in mice deficient in both IL-4 and IL-10, was associated with reduced hepatic steatosis, serum transaminase levels and liver fibrosis. These protective effects seemed to be mediated by increased levels of IFNγ, as use of *Ifng*$^{-/-}$ mice resulted in more severe liver injury and liver fibrosis than in wild-type mice. *Ifng*$^{-/-}$ mice also had higher levels of transforming growth factor β (TGFβ) expression, although use of a TGFβ-blocking antibody resulted in only modest reductions in fibrosis. This effect was believed to be because TGFβ blockade was associated with an increase in eosinophil infiltration and a type II inflammatory response. Notably, dual TGFβ and IL-13 blockade (a cytokine associated with type II inflammatory responses) in mice produced a more substantial reduction in fibrosis

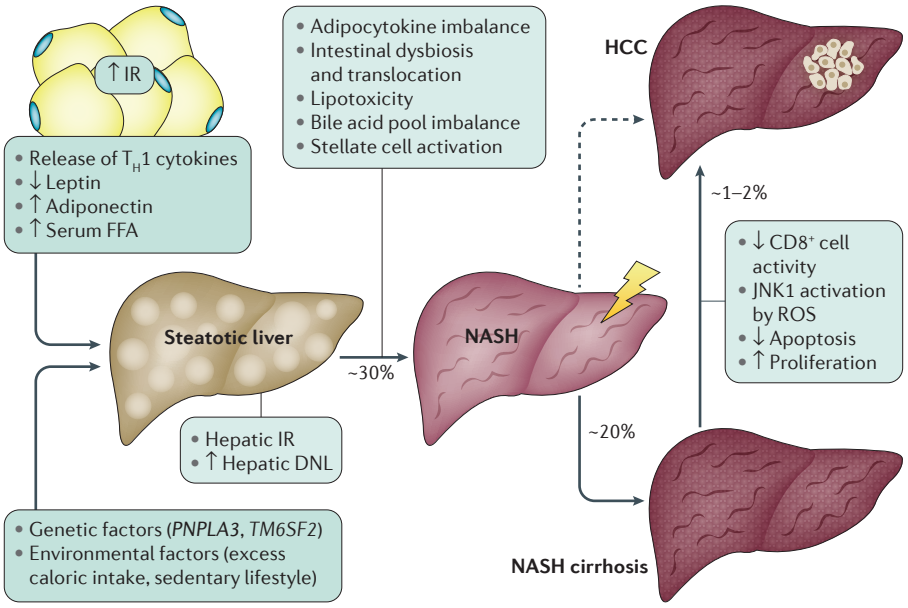

Figure 1 | **NAFLD pathogenesis.** A combination of genetic factors and environmental factors can influence hepatic steatosis risk. In patients with hepatic steatosis, inflammation is triggered by multiple mechanisms. These processes activate Kuppfer cells and stellate cells, and promote a type 2 T helper (T_H2) cell inflammatory response, with progressive fibrosis. NASH can progress to cirrhosis. Hepatocellular carcinoma (HCC) often occurs in the context of NASH cirrhosis but can occur without cirrhosis. Inhibition of activated $CD8^+$ T cells and increased cell proliferation confer increased HCC risk. DNL, *de novo* lipogenesis; FFA, free fatty acids; IR, insulin resistance; JNK1, c-Jun N-terminal kinase 1; ROS, reactive oxygen species.

Key advances

- Neuregulin 4 (NRG4), released by adipocytes, inhibits degradation of cellular FADD-like apoptosis regulator (CFLAR), which protects against hepatocyte death; a reduction in hepatic NRG4 levels represents a potential checkpoint for progression of hepatic steatosis to NASH[4]
- Type II inflammatory responses work in conjunction with transforming growth factor β (TGFβ) to mediate fibrosis progression in NAFLD[6]
- Patients with NAFLD have increased levels of IgA$^+$ cells in the liver, which are associated with an increased risk of hepatocellular carcinoma via inhibition of CD8$^+$ T cell activity[9]

than TGFβ blockade alone. This study suggests that type II inflammatory responses could be key drivers of NAFLD disease progression, and that in view of the multiple immunological effects of blocking individual cytokines, treatments that target a number of mechanisms simultaneously might be more effective in reducing fibrosis. However, the potential of TGFβ blockade to counteract liver fibrosis must be approached with caution, as TGFβ can act as either a tumour suppressor or a tumour promoter depending on the microenvironment[7].

Patients with NAFLD are at increased risk of developing HCC, with the incidence of NAFLD-associated HCC rising 9% per year[1]. Currently, the only effective treatment for HCC is resection or ablation for localized disease. The first line drug treatment for HCC is sorafenib, a small-molecule kinase inhibitor that prolongs survival by a few months compared with placebo[8], leaving a clear unmet need for new treatments. Although immunotherapy is licensed for treatment of several malignancies, the role of the immune system in cancer development is a matter of debate.

In 2017, work by Shalapour *et al.*[9] shed light on the role of activated immune cells in HCC development. They found that patients with NASH fibrosis had higher levels of IgA$^+$ plasma cells in the liver compared with patients without NASH fibrosis, and that these cells were phenotypically and functionally distinct from intestinal IgA$^+$ B cells. Hepatic IgA$^+$ cells expressed programmed cell death 1 ligand 1 (PD-L1) and IL-10, which both inhibit CD8$^+$ T-cell activity, with IgA-deficient mice having a lower incidence of HCC. However, ablation of CD8$^+$ cells in these mice restored HCC development. Finally, use of a PD-L1 antibody reduced tumour multiplicity and load independently of serum IgA levels. Therefore, these findings suggest that CD8$^+$ cells have an important role in preventing HCC emergence, and strategies that augment CD8$^+$ cell activity could be used in HCC prevention or treatment. In 2017, the results of the first (nonrandomized) phase I/II clinical trial of a PD-L1 inhibitor, nivolumab, in patients with advanced HCC were published[10]; the treatment was well-tolerated, with some evidence of efficacy. A phase III randomized, controlled trial is currently under way.

In summary, a better understanding of disease pathogenesis has resulted in a number of novel emerging therapeutic targets for the prevention and treatment of NAFLD. This success has only been possible due to the development of effective mouse models of disease that closely emulate disease progression in humans. The challenge now is to establish whether these treatments can make the jump from the bench to the bedside and improve outcomes for this common condition.

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Competing interests statement
P.N.N. has served as a speaker, a consultant and an advisory board member for AbbVie, Boehringer Ingelheim, Gilead, Intercept, Janssen, Novartis, Novo Nordisk, Pharmaxis and Shire, and has received research funding from these organizations on behalf of the University of Birmingham, UK. R.S.K. has received research funding from the National Institute of Health Research.

IBD IN 2017

Development of therapy for and prediction of IBD — getting personal

Raja Atreya and Britta Siegmund

The central studies published in 2017 address novel IBD therapeutic strategies and prediction of the future disease course or response to a distinct therapy. Together, these studies contribute to the understanding of the regulation of mucosal homeostasis and at the same time serve to develop novel personalized treatment algorithms in patients in whom a severe disease course can be predicted.

IBD represents chronic remittent disorders that have been characterized by an increasing incidence over the past six decades with only an incompletely understood pathogenesis and yet the need to provide a cure. Data from the past century suggest that about one-quarter of the occurring disease cases derive from the presence of risk mutations, of which variants of *NOD2* present the best known example[1]. However, the striking increase in incidence points towards a contribution by environmental factors. The current concept of pathogenesis has developed to include the close interplay between the intestinal microbiota, the epithelial barrier and the mucosal immune compartment in determining gut homeostasis.

Hence, it seems intriguing to develop a therapeutic strategy by resetting one of these three pathogenic players (FIG. 1). One example of this approach, based on earlier research and the associated difficulties of identifying the 'ideal' donor, is the 2017 study by Paramsothy *et al.*[2] in which faecal microbiota transplantation (FMT) was performed by applying a multi-donor transplant combined with a high intensity of FMT (five times per week for a total of 8 weeks). The combined primary end point (steroid-free clinical remission and endoscopic remission or response) was met, pointing towards the feasibility of this luminal-focused approach[2]. Nevertheless, future work will have to answer numerous questions, including the ideal composition of FMT material and the substitutability of human donor faecal samples for a fully laboratory-derived and defined transplant[3].

> "…a well-defined set of tissue markers will potentially enable clinicians to allocate optimal treatment to the individual patient…"

On the mucosal domain, TNF antibodies have been dominating IBD therapy for the past 17 years. In 2016, the second anti-cytokine-based antibody, ustekinumab, received FDA approval for Crohn's disease[4]. Ustekinumab targets the subunit p40 (a subunit of both IL-12 and IL-23) and results in a neutralization of two pro-inflammatory mediators, IL-12 and IL-23. This aspect poses the question of whether or not it would be sufficient to target either one of those two mediators with the same outcome of successfully treating inflammation in IBD. In line with earlier experimental data suggesting that sole inhibition of IL-23 might suffice to ameliorate intestinal inflammation, two phase II studies published in 2017 underline this concept. The first randomized, double-blind, placebo-controlled phase II study randomly assigned patients with Crohn's disease with a moderate-to-severe disease activity to either risankizumab (either 200 or 600 mg), a humanized monoclonal antibody targeting the p19 subunit of IL-23, or placebo at weeks 0, 4 and 8.93% of the patients had previously been exposed to anti-TNF treatment (26% with an inadequate response and 54% with a loss of response). The primary end point of clinical remission at week 12 was met by 31% (including pooled data from the 200 and 600 mg arm) of the risankizumab group versus 15% of the placebo group[5]. The second phase IIa trial included patients with moderate-to-severe Crohn's disease who had failed treatment with anti-TNF antibodies, with the results indicating that treatment with the p19-antibody MEDI2070 for either 8 or 24 weeks was, like risankizumab, also associated with clinical improvement[6].

Importantly, the antibodies against p40 and p19 subunits represent only a selection of IBD therapeutic strategies under development. This luxurious vision that multiple options are available for the treatment of our patients leads to the central issue, somewhat comparable to the FMT donor question discussed earlier, of identifying the most suitable patient for a defined treatment strategy. Two studies from 2017 provide some insight into this question.

The first study by West *et al.*[7] showed that patients with IBD express high amounts of the IL-6 family member oncostatin M (OSM) in the inflamed intestine, which correlates with disease activity. The production of OSM is followed by the release of pro-inflammatory mediators, including IL-6, intercellular adhesion molecule 1 (ICAM1) and chemokines, ultimately inducing the infiltration of neutrophils, monocytes and T cells into the intestine. Remarkably, the blockade or genetic deletion of OSM ameliorated colitis in a mouse model resistant to anti-TNF therapy. Hierarchical clustering of 200 patients with IBD (cohorts from two phase III clinical trials of infliximab and golimumab) identified that high intestinal tissue expression of OSM before anti-TNF treatment was strongly associated with failure of anti-TNF treatment. Although these data warrant a prospective validation, OSM might turn out to be a predictive marker for anti-TNF therapy. The association of the local expression of a distinct mediator here is in line with earlier data in which high mucosal TNF expression could be linked to the response to anti-TNF treatment[8].

In the end we might need a set of distinct locally expressed markers that will enable stratification to match available therapies. Although this strategy is attractive, it would be equally helpful to be able to predict the prognosis of the disease course of each individual patient. We are all aware of the substantial variations in disease course that occur when we follow our patients. A 2017 study aimed to characterize how genetic variation influences disease prognosis[9]. To address this issue, a within-cases genome-wide association study including two cohorts with Crohn's disease was performed. Contrary to the hypothesis that disease susceptibility and prognosis share the same genetic architecture[10], four genome-wide significant loci were identified, none of which were associated with disease susceptibility but were associated with prognosis. The four association signals were located in *FOXO3*, *XACT*, a region upstream of *IGFBP1*, and the major histocompatibility complex (MHC) region. Only limited data are available on the mechanistic effects of these four loci on disease pathogenesis: *FOXO3* is involved in the

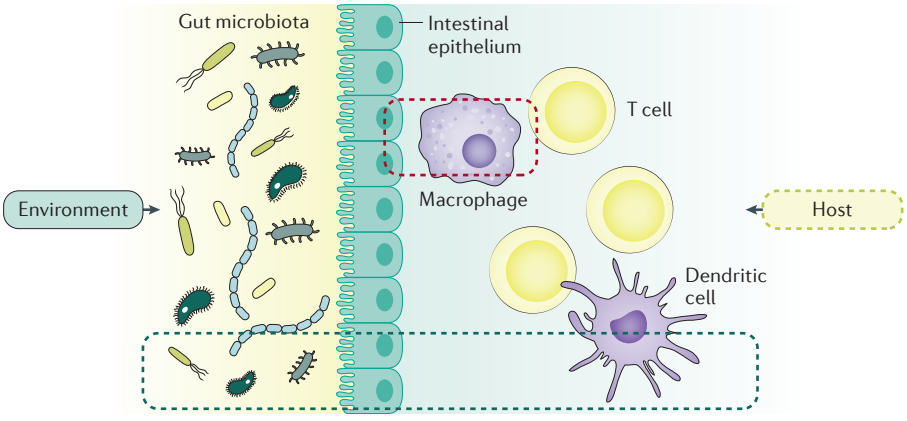

Figure 1 | **The complex interplay between environment and host in IBD.** The environment is represented by the gut microbiota, intestinal barrier and the immune cell composition in the lamina propria. Different elements of this interplay will serve to develop novel therapeutic approaches, personalized strategies or even enable prediction of the disease course. FMT, faecal microbiota transplantation; mTNF, membrane-bound TNF; OSM, oncostatin M.

Key advances

- Repetitive, multi-donor faecal microbiota transplantation induces steroid-free clinical and endoscopic remission in patients with active ulcerative colitis[2]
- Selective inhibition of the p19 subunit of IL-23 leads to substantial clinical improvement in patients with moderate-to-severe Crohn's disease who had prior non-response to anti-TNF treatment[5,6]
- High pretreatment tissue expression of the IL-6 family member oncostatin M is predictive of failure to respond to subsequent anti-TNF therapy in patients with IBD[7]
- Four genome-wide significant loci are identified as genetic variants associated with disease prognosis, but not disease susceptibility, in Crohn's disease[9]

regulation of the transforming growth factor-β pathway and inhibits inflammatory responses in monocytes. *XACT* is only expressed on the active X chromosome and has been studied in pluripotent stem cells; beyond this the function is unknown. *IGFBP1* has been associated with immunity and longevity. In addition, the locus has been linked to antibodies to citrullinated peptides in rheumatoid arthritis. Within the MHC region, all associated alleles belonged to the 'ancestral MHC 8.1' haplotype and could previously have been linked to impaired responses to vaccination and T cell activation.

The detailed functional analysis of those four loci will be essential for the understanding of the disease pathways. Importantly, this study emphasizes that we will need to distinguish between disease susceptibility and biology of disease prognosis in our mechanistic studies, since the respective genetic drivers differ. Ultimately, this discrimination between disease susceptibility and prognosis might add an additional layer to a future therapeutic algorithm model. At this point, the study has only included patients with Crohn's disease, therefore, the analysis will need to be performed for ulcerative colitis as well to generalize this concept. In addition, a prospective cohort is desirable to establish and confirm this novel concept.

Predicting the next 10 years of IBD research, we will hopefully be able to determine the disease course at diagnosis, possibly through genetic testing. Reflecting the thoughts about disease susceptibility discussed earlier, we might learn over the next decade that environmental factors also independently alter the prognosis. In addition, a well-defined set of tissue markers will potentially enable clinicians to allocate optimal treatment to the individual patient and, therefore, finally establish personalized medicine for the field of IBD.

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Competing interests statement
R.A. served as consultant for Abbvie, Janssen and Pfizer, and received speaker's fees from Abbvie, Falk, Janssen, MSD and Takeda. B.S. received a research grant from Pfizer, served as consultant for Falk, Janssen, MSD, Abbvie, Takeda and Hospira, and received lecture fees from Abbvie, Falk, Ferring, MSD, Merck and Takeda; all money went to the Charité–Universitätsmedizin Berlin, Germany.

HEPATOCELLULAR CARCINOMA IN 2017

Two large steps forward, one small step back

Marcus-Alexander Wörns and Peter R. Galle

In 2017, the FDA approved regorafenib and nivolumab for the treatment of patients with hepatocellular carcinoma following prior sorafenib treatment, opening the door for an effective systemic second-line therapy in advanced disease. By contrast, the addition of sorafenib to transarterial chemoembolization with drug-eluting beads did not improve progression-free survival in the intermediate disease stage.

Globally, hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death as most patients are diagnosed at advanced stages and, in addition, suffer from underlying liver cirrhosis, which further limits treatment options. The Barcelona Clinic Liver Cancer (BCLC) classification is the most widely used staging system in Western countries for estimating patient prognosis and choosing the appropriate treatment in consideration of the disease stage[1]. In the heterogeneous intermediate stage (BCLC B; multifocal disease, confined to the liver, maintained liver function and performance status), transarterial chemoembolization (TACE) might provide survival benefit in selected patients[2]. For patients with advanced disease (BCLC C; presence of macrovascular invasion (MVI) and/or extrahepatic spread (EHS), and/or impaired performance status), the multikinase inhibitor sorafenib — which targets angiogenesis and tumour proliferation — was the first systemic agent to provide statistically significant improvement in overall survival (OS), in a study published almost ten years ago (10.7 versus 7.9 months for sorafenib versus placebo (HR 0.69; 95% CI 0.55–0.87, $P < 0.001$))[3]. However, all subsequently tested agents failed in phase III randomized controlled trials (RCTs), both in first-line and second-line settings, not reaching or improving the magnitude of benefit obtained with sorafenib (first line) or even placebo (second line)[4]. As a consequence, there has been no standard therapy during the past ten years for patients with disease progression on sorafenib treatment.

2017 saw the publication of the RESORCE trial, which assessed another multikinase inhibitor, regorafenib, as second-line therapy in patients with HCC. Molecularly similar but pharmacologically distinct from sorafenib, regorafenib improved OS compared with placebo in patients with HCC who had progressed on sorafenib treatment (10.6 months for regorafenib versus 7.8 months for placebo (HR 0.63; 95% CI 0.50–0.79; $P < 0.0001$))[5]. The success of this sequential approach using two multikinase inhibitors was in part due to the fact that patients must have tolerated sorafenib, which avoided unexpected toxicity. Furthermore, the optimized study design with intensive stratification (for the first time separating patients with MVI and EHS), the assumption of a median OS of 8 months in the placebo group for statistical analysis, and strict inclusion criteria including well-preserved liver function (Child–Pugh A) and maintained performance status contributed to success. However, the strong patient selection might present a potential limitation of this pivotal RCT when translated into real-world settings, and it remains to be seen what percentage of patients with HCC will ultimately benefit from this second-line option. In 2017, the FDA and the European Medicines Agency (EMA) expanded the approved use of regorafenib (in colorectal cancer and gastrointestinal stromal tumours) to include treatment of patients with HCC who have been previously treated with sorafenib. Regorafenib will become standard of care as second-line treatment in advanced HCC, most probably in patients who progressed on and tolerated prior sorafenib treatment and are still fit for systemic therapy.

> “...the results of CheckMate040 will considerably affect the future landscape of systemic treatment in HCC...”

Nevertheless, unmet needs in advanced HCC will still exist, foremost in patients not tolerating sorafenib or regorafenib or progressing on both drugs. The success of immune oncology in other tumour entities has also encouraged implementation of immunotherapeutics in HCC. Administration of checkpoint inhibitors reactivates the exhausted antitumour response of cytotoxic T cells, which results in objective and often durable responses in a sizeable percentage of patients. In 2017, this effect was shown with the monoclonal antibody nivolumab, a programmed cell death protein 1 (PD-1) inhibitor, in a large phase I/II, open-label, dose-escalation and dose-expansion study (the CheckMate040 trial) in patients with advanced HCC and maintained liver function (Child–Pugh A to B7)[6]. Aside from the manageable toxicity profile without new safety signals, including in patients with viral hepatitis, this study in patients with advanced disease (68% EHS) and frequent prior systemic treatment (76%, mostly sorafenib) showed strong signals of nivolumab antitumour activity. The objective response rates (15–20%) and OS benefits (median OS 28.6 months in patients naive to sorafenib, median OS 15.0 months in patients previously treated with sorafenib) were unseen in previous trials in HCC[7]. Despite the non-randomized study design and the absence of an association between nivolumab response and tumour cell PD-1 ligand 1 (PD-L1) expression, the results of CheckMate040 will considerably affect the future landscape of systemic treatment in HCC, as already reflected by the decision of the FDA to grant an accelerated approval to nivolumab for the treatment of patients with HCC following prior sorafenib therapy. Thus, in addition to regorafenib, nivolumab offers another second-line option after sorafenib treatment. Results of an ongoing phase III RCT comparing nivolumab with sorafenib head to head in the first-line setting (CheckMate459, NCT02576509) will clarify the role of nivolumab in patients with advanced HCC.

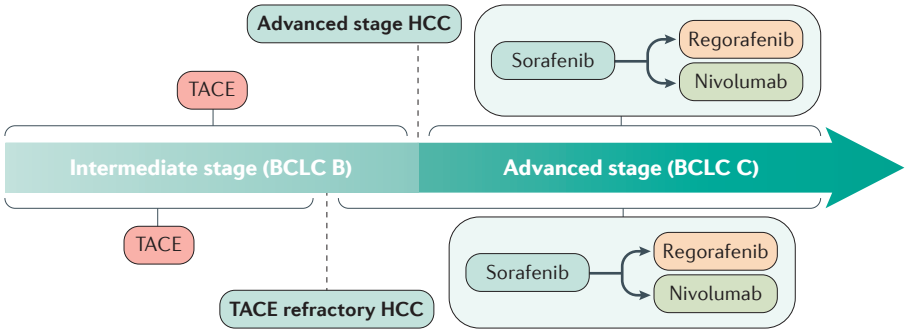

Figure 1 | **Sequential treatment requires a timely switch from transarterial approaches to systemic treatment in patients with well-preserved liver function.** This switch takes place at the intermediate stage when patients become transarterial chemoembolization (TACE) refractory (bottom) or in the advanced stage as standard of care (top). However, the clinical reality is that this switch continues to be a challenge and many patients do not receive systemic therapy. BCLC, Barcelona Clinic Liver Cancer stage; HCC, hepatocellular carcinoma.

For patients with intermediate HCC, TACE is recommended by most international guidelines[2] and TACE (either as conventional TACE or with drug-eluting beads (DEB–TACE)) is standard of care worldwide. However, most patients (even with selection) present with disease relapse or experience disease progression after TACE, with a median life expectancy of ~2 years in clinical practice[2]. One potential explanation is that incomplete necrosis and hypoxia in TACE-treated lesions leads to a cytokine storm, which might contribute to rebound neovascularization and metastasis. This effect provides the rationale for combining TACE with an antiangiogenic agent, such as sorafenib. A phase II RCT (the SPACE trial) comparing DEB–TACE plus sorafenib with DEB–TACE plus placebo formally met its primary end point of improved time to progression (TTP) and was published in 2016; however, TTP differences between arms were only marginal and considered clinically irrelevant[8]. The phase III TACE 2 RCT reported in 2017 that the concurrent administration of sorafenib and DEB–TACE did not improve progression-free survival, disease response rates or OS in patients with intermediate HCC and maintained liver function (Child–Pugh A)[9]. These negative results draw the final curtain over the clinical implication of the concurrent administration of DEB–TACE and antiangiogenic agents (for example, sorafenib or brivanib) in this stage of the disease.

The approval of regorafenib and nivolumab enlarges our therapeutic armamentarium for patients with advanced HCC. However, the low objective response rates demand further improvement, which might be achieved by exploiting potential synergistic strategies, particularly the combination of checkpoint inhibitors with targeted therapies. In addition, after unsuccessful trials investigating the addition of sorafenib to TACE or sorafenib in an adjuvant setting after potentially curative resection or local ablation, the rationale is clear to explore the complementary role of checkpoint inhibitors in combination with TACE in intermediate stages, or in an adjuvant setting after potentially curative resection or local ablation in early stages.

Key advances

- Regororafenib will become standard of care as systemic second-line therapy in hepatocellular carcinoma (HCC) after sorafenib treatment[5]
- Nivolumab might be another second-line option after sorafenib treatment, with objective response rates and overall survival (OS) benefits unseen in previous trials in HCC[6]
- The addition of sorafenib to drug-eluting bead transarterial chemoembolization does not improve OS in intermediate HCC[9]

Real-life practice in the past demonstrated an exaggerated usage of TACE and a neglect of systemic therapy. As exploratory analyses of the sequential use of sorafenib and regorafenib (median OS 26.0 months[10]) or with nivolumab in patients naive to sorafenib (median OS 28.6 months[7]) showed a clinically meaningful OS benefit, it is likely that an increased percentage of patients with intermediate and advanced disease will receive systemic therapy in the future. This appraisal is gaining additional support from further positive trials that have not yet been published (lenvantinib (NCT01761266) and cabozantinib (NCT01908426) in first-line and second-line settings, respectively).

In summary, 2017 saw us move two large steps forward, and systemic therapy of HCC is becoming multifaceted. The decision to switch in a timely manner from transarterial approaches to systemic treatment in patients with well-preserved liver function is mandatory to avoid passing the point of no return (FIG. 1), after which poor liver function prevents systemic therapy.

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PANCREATIC CANCER IN 2017

Rebooting pancreatic cancer knowledge and treatment options

Alexander Semaan and Anirban Maitra

High stromal cellularity in pancreatic cancer is an important factor for ineffective treatment and molecular studies. In 2017, major advancements were made in transcriptional characterization, treatment delivery and clinical regimes, raising hope for a breakthrough against this deadly disease.

Pancreatic ductal adenocarcinoma (PDAC) is one of the few solid cancers with a rising incidence globally and is predicted to become the second most common cause of cancer-related deaths within the next decade in the United States[1]. Major causes of lethality are the late detection of PDAC due to nonspecific symptoms at early stages of the disease, the absence of effective screening modalities and the lack of potent systemic therapies.

Despite these overarching challenges, the past decade has seen substantial advancements in our understanding of the molecular underpinnings and risk factors associated with PDAC, partly based on genetically engineered animal models[1]. Advances in pancreatic cancer have also extended beyond the laboratory, with the approval of two new first-line regimens (FOLFIRINOX and gemcitabine plus albumin-bound paclitaxel) for metastatic disease that have improved patient survival. Each year, we have seen publications of the highest quality in both the research and clinical arenas that harbour the potential for significant impact on the course of this disease, and 2017 has been no different.

The advent of next-generation sequencing (NGS) technologies has enabled unprecedented insight into the molecular landscapes of most solid cancers, facilitated by large-scale, publicly funded efforts such as the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). The ICGC published the first NGS-based profiling study of the PDAC genome, which was updated in 2016 with the addition of transcriptomic data and the development of a four-tier mRNA-based classification system reflecting apparently distinct tumour biology[2]. 2017 saw the publication of the corresponding TCGA effort in PDAC, which comprised an integrated and multiplatform characterization of 150 human PDAC samples[3]. The multiplatform nature of the TCGA analysis was underscored by inclusion of data not only from the whole exome and coding RNA, but also the epigenome (methylome), non-coding RNA (both long non-coding RNA and microRNA) and array-based proteomics, all of which were then integrated using computational algorithms for biological patterns. In particular, the TCGA team needs to be commended for developing tools (including the so-called ABSOLUTE algorithm) that digitally overcame the challenges of low neoplastic cellularity and high stromal heterogeneity in PDAC. These tools have helped clarify that the previous four-tier mRNA classification from the ICGC is likely a reflection of suboptimal cellularity (the relative proportion of (few) tumour and (many) stromal cells), and that PDAC can be essentially divided transcriptionally into two subtypes — basal-like–squamous and classic–progenitor — each with distinct biological underpinnings and mechanisms of gene regulation.

The TCGA analysis reiterated that mutant *KRAS* remains the dominant oncogene in PDAC, with 93% of samples demonstrating

mutations in this gene. Notably, even in the few *KRAS* wild-type samples, 60% harboured a mutation in components of the canonical RAS–MAPK (mitogen-activated protein kinase) pathway or upregulation of mechanistic target of rapamycin (mTOR) protein, a distal effector of RAS, reiterating the importance of this signalling pathway in pancreatic carcinogenesis. Other previously identified driver genes, such as those involved in homologous DNA repair (*BRCA2*, *ATM* and *PALB2*), and epigenetic regulation (*ARID1A*, *PBRM1* and *KDM6A*), were confirmed and a new driver gene, *RREB1*, was catalogued. The importance of defects in homologous DNA repair pertains to the susceptibility of the corresponding tumours to platinum agents or a new class of inhibitors that inhibit a parallel DNA repair pathway catalysed by poly [ADP-ribose] polymerase (PARP). Inhibitors of PARP have shown demonstrable success in ovarian and breast cancers with DNA repair defects[4], and are now being evaluated in PDAC. Needless to say, the wealth of publicly available profiling data and bioinformatics tools generated by the TCGA will facilitate numerous secondary analysis studies related to the biology and prognosis of PDAC for years to come. Thus, selecting this publication as a 2017 highlight was a foregone conclusion.

Given the propensity for a near-ubiquitous activating point of *KRAS* in PDAC, it is not a surprise that this oncogene represents a compelling therapeutic target. Unfortunately, drug development against RAS, a membrane-tethered small GTPase, has been challenging, with prior attempts such as farnesyltransferase inhibitors largely failing in the clinic. In 2017, small-molecule inhibitors against the *KRAS*^{G12C} allele have been described[5], but these agents rely on covalent binding to the Cys12 moiety, and therefore lack efficacy in the *KRAS*^{G12D} and *KRAS*^{G12V} alleles that comprise the overwhelming majority of mutations in PDAC. Genetic approaches to RAS inhibition have been developed using small interfering RNAs (siRNA) specifically directed to the mutant allele, but in preclinical models the delivery of these molecules using lipid nanoparticle or liposomal platforms has been variably efficient[6]. However, in 2017, Kamerkar *et al.*[7] developed a novel systemic approach for delivery of siRNA against *KRAS*^{G12D}, wherein the genetic cargo was loaded by electroporation into so-called inhibitory exosomes (iExosomes) derived from human foreskin fibroblasts. The authors demonstrated the superiority of iExosomes in attenuating tumour growth compared with liposomal formulations in multiple preclinical *in vivo* PDAC models, including orthotopic xenografts, syngeneic allografts and patient-derived xenografts, with some of the experiments also measuring increased median survival of the animals as an end point. The observed higher systemic antitumour efficacy compared with liposomes might be at least partly attributed to the expression of a 'don't eat me' signal (CD47) on the surface of natural exosomes, which bypasses uptake by the reticuloendothelial system[8]. Moreover, PDAC remains an attractive tumour type for iExosome-based delivery as these particles enter neoplastic cells via a primitive nutrient uptake mechanism known as macropinocytosis, which is greatly enhanced in the presence of an oncogenic RAS[9]. The path from preclinical studies in mice to clinical trials in humans is arduous and will require toxicity and biodistribution studies in larger animals, adapting to an intravenous modality for the clinic, as well as scaling up and addressing the regulatory challenges of iExosome production. Despite these caveats, this study represents an important step towards therapeutic targeting of the 'undruggable behemoth' that is *KRAS*.

The final highlighted paper for 2017 is one that has already altered clinical practice. Although ~85% of patients with PDAC are deemed surgically unresectable[1], it is the minority that do undergo surgery where the potential for prolonged survival has been most frequently realized. This understanding has occurred, in part, due to improvements in surgical techniques, greater acceptance of neoadjuvant therapy and improvements in postoperative (adjuvant) regimens. Patients who successfully undergo R0 (margin negative) resection with appropriately administered adjuvant therapy have median survival rates of 20 months or more. In 2004, the landmark European Study Group for Pancreatic Cancer (ESPAC-1) trial established the superiority of adjuvant 5-flurouracil (5-FU) compared with observation alone in resected PDAC. In the latest iteration of ESPAC (ESPAC-4), Neoptolemos and colleagues compared the combination of gemcitabine plus capecitabine versus gemcitabine alone in the adjuvant, post surgical setting, conducting a randomized clinical trial of >700 patients with PDAC[10]. They found significant improvement in the median survival of patients receiving the combination therapy compared with those receiving gemcitabine alone

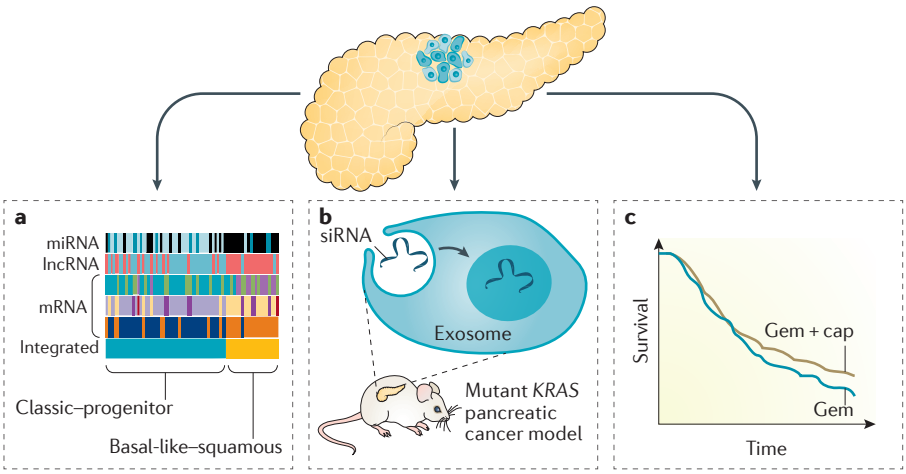

Figure 1 | **Selected advances in pancreatic ductal adenocarcinoma during 2017.** Studies highlighted here include an integrated characterization of the molecular landscape of pancreatic ductal adenocarcinoma (PDAC) from The Cancer Genome Atlas (part **a**), which confirmed the existence of two subtypes based on molecular expression profiles with prognostic implications; a novel systemic delivery platform using inhibitory exosomes (iExosomes) to genetically target mutant *KRAS* in multiple preclinical PDAC models (part **b**); and a new standard of care, gemcitabine plus capecitabine, showed improved overall survival for the adjuvant therapy of resected PDAC (part **c**). lncRNA, long non-coding RNA; miRNA, microRNA; siRNA, small interfering RNA.

Key advances

- Integrated analysis of the pancreatic ductal adenocarcinoma (PDAC) genome, epigenome and transcriptome revealed a complex molecular landscape, showing the currently 'undruggable' *KRAS* as the predominant oncogenic driver, plus distinct subtypes with prognostic implications[3]
- Engineered exosomes loaded with small interfering RNA and short hairpin RNA specific to *KRAS*^{G12D} showed promising preclinical results in the treatment of *KRAS*-mutant PDAC, providing one possible approach to narrowing the druggability gap against this oncogenic driver[7]
- In surgically resected patients with PDAC, adjuvant gemcitabine plus capecitabine demonstrated an improved 5-year overall survival rate over gemcitabine monotherapy in a large randomized phase III trial, establishing a new standard of care[10]

(28 months versus 25.5 months, $P = 0.032$). Importantly, although increased survival conferred by the combination therapy was observed across both R0 (margin negative) and R1 (margin positive) subgroups, the results were most profound (an almost 12 month difference in median survival; ~27 months versus ~39 months) in patients with R0 margin status. Another compelling prognostic effect was observed in the long-term follow up data, with ~29% of patients surviving to 5 years or beyond in the combination arm versus ~16% in the gemcitabine subgroup. Based on these results, gemcitabine plus capecitabine is now accepted as one of the standards for adjuvant therapy in patients with PDAC worldwide. ESPAC-4 has unequivocally presented a new and improved option for oncologists in the adjuvant care of patients with PDAC.

In summary, this past year presented the PDAC clinical and research community with exciting translational and clinical advances (FIG. 1). The selection of aforementioned studies represents a mere snapshot of the vibrant activity ongoing in this disease, in which we continue to make progress despite obvious daunting challenges.

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Competing interests statement
The authors declare no competing interests.

STEM CELLS IN 2017

Digesting recent stem cell advances in the gut

Nick Barker

2017 has witnessed major advances in gut stem cell and cancer stem cell research, delivering key insights into their regulation, more defined culture methods and novel stem cell markers that collectively drive us ever closer to breakthroughs for regenerative medicine and cancer treatment in the clinic.

The epithelial lining of our gut operates in a hazardous environment that curtails the lifespan of the constituent cells, imposing a requirement for daily epithelial renewal. Characterization of the stem cells responsible for this massive renewal effort has accelerated over the past decade, driven by the development of *in vivo* lineage tracing and cell ablation models and near-physiological culture technologies. This rapid progress has continued in 2017, with the discovery of new stem cell populations in the stomach, demonstration of cancer stem cell (CSC) plasticity in colorectal cancers (CRCs) and the development of defined, synthetic culture matrices compatible with human use (FIG. 1).

A detailed understanding of the local microenvironment or niche that regulates gut stem cells is of vital importance if we are to safely culture stem cells and exploit their regenerative potential in the clinic. In the intestinal crypts, activation of WNT signalling is essential for maintenance of stem cell-driven epithelial homeostasis[1]. One consequence of this WNT signalling is the induction of leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) expression on intestinal stem cells (ISCs), endowing them with the ability to bind R-spondin (RSPO) ligands and block the default negative feedback loop designed to limit WNT signalling through the actions of the membrane-bound E3 ubiquitin–protein ligases ZNRF3 or RNF43. This LGR5–RSPO signalling ensures that WNT signal strength is amplified to a level compatible with optimal stem cell function.

In 2017, additional insight into the complex interplay between WNT and RSPO signalling was provided using an elegant set of tools to dissect their relative contributions to the regulation of crypt homeostasis and intestinal stem cells in mice[2]. Adenovirus systems were used to deliver: a novel synthetic WNT anologue that crosslinks frizzled (FZD) and low density lipoprotein receptor-coupled protein (LRP) co-receptors at the cell surface to hyperactivate WNT signalling[3]; a fragment of the WNT co-receptor FZD8 to block WNT signalling; or the RSPO-binding ectodomains of LGR5, RNF43 or ZNRF3 to block endogenous RSPO–LGR5 signalling. Interestingly, Yan *et al.*[2] found that WNT and RSPO are not functionally equivalent in the intestine. Although both were required for maintenance of ISCs and long-term epithelial homeostasis in the intestine, *in vivo* rescue experiments identified WNT as the major effector of intestinal crypt homeostasis. However, the level of RSPO signalling was found to be the critical determinant of stem cell pool size as a consequence of its role in regulating the balance between ISC self-renewal and differentiation. The primary function of WNT signalling in the intestine is, therefore, to endow the ISC compartment with the ability to activate the LGR5–RSPO signalling axis that controls ISC self-renewal.

> “...stem cell activity driven by RSPO3 is an important mechanism for limiting colonization by pathogenic bacteria”

Similar bimodal WNT–RSPO signalling mechanisms might regulate the activity of other LGR5$^+$ stem cell or CSC populations. A separate 2017 study identified RSPO3, expressed by myofibroblasts, as a critical modulator of WNT-responsive stem cells in the lower regions of antral glands in the stomach[4]. Conditional knockout of endogenous RSPO3 reduced expression of WNT target

genes in the stomach epithelium, including LGR5. However, in contrast to the observations made in the intestine, overexpression of RSPO1/3 in mice had no effect on either the size or activity of the LGR5$^+$ stem cell pool at the gland base[4]. Instead, increased RSPO signalling was postulated to transiently increase the activity of an LGR5-independent population of WNT-responsive stem cells marked by AXIN2, which are present in the lower isthmus region of gastric glands. Notably, infection by *Helicobacter pylori*, a potent gastric carcinogen, triggered an increase in local RSPO3 expression with a concomitant increase in stem cell activity[4]. Conditional knockout of RSPO3 enhanced *H. pylori* infection in mice, prompting speculation that increased stem cell activity driven by RSPO3 is an important mechanism for limiting colonization by pathogenic bacteria.

In the acid-secreting corpus region of the stomach, Matsuo *et al.* recently identified runt-related transcription factor 1 (RUNX1) as a marker of proliferative isthmus cells and rare zymogenic chief cells at the gland base[5]. *In vivo* lineage tracing driven by RUNX1 promoter elements demonstrated that isthmus-resident RUNX1$^+$ cells function as multipotent stem cells during homeostasis. In the antrum, RUNX1 expression was confined to the gland base, overlapping with the established LGR5 stem cell zone. Zymogenic chief cells in the stomach corpus are thought to demonstrate functional plasticity following epithelial injury or oncogenic transformation. Formal proof of this model was provided this year by Leushacke *et al.*, in which LGR5 expression was found in a subpopulation of chief cells at the gland base[6]. Using a new LGR5-Cre mouse model, *in vivo* lineage tracing was used to document these LGR5$^+$ chief cells as damage-inducible stem cells effecting long-term gland regeneration following injury. Importantly, constitutive activation of KRAS in these reserve stem cells initiated spasmolytic polypeptide-expressing metaplasia, a form of metaplasia considered to be a precursor of gastric cancer in humans, confirming that chief cells can serve as a cancer cell origin[6].

Key advances

- WNT and R-spondin (RSPO) signalling are not functionally equivalent in the intestinal stem cell niche; WNT signalling primes stem cells for RSPO signalling, which controls stem cell population size and activity[2]
- RSPO3 regulates WNT-responsive stem cell activity in the antral stomach and modulates *Helicobacter pylori* infection[4]
- Colon cancers exhibit cancer stem cell plasticity, but liver metastases are unable to survive loss of their endogenous leucine-rich repeat-containing G-protein coupled receptor 5$^+$ (LGR5$^+$) cancer stem cells, which has important implications for treating colon cancer[9,10]

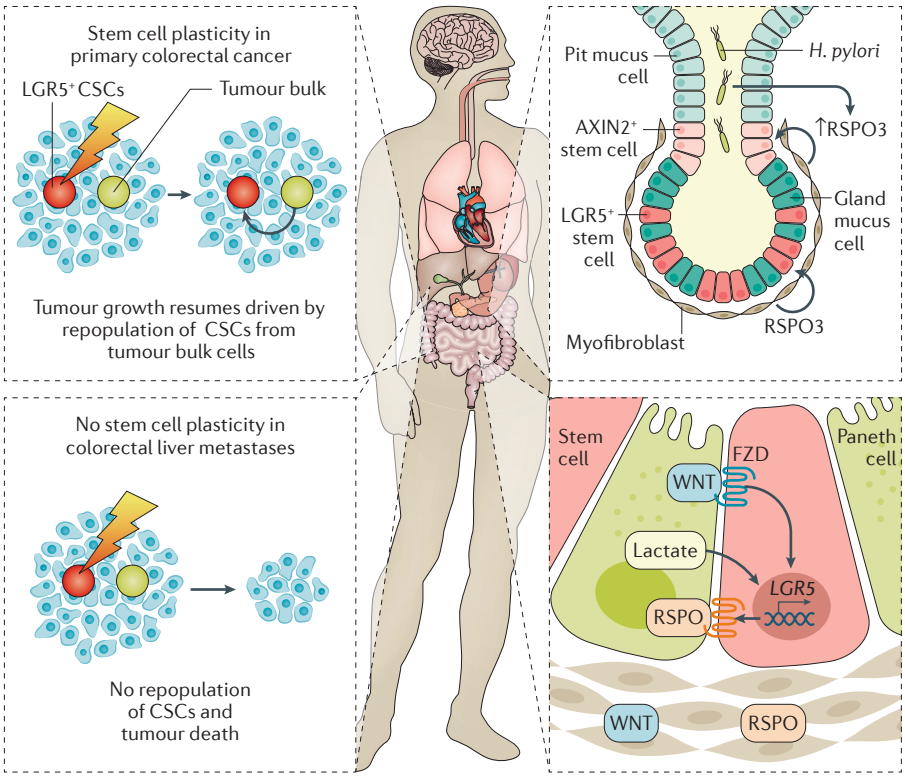

Figure 1 | **Cancer stem cell plasticity and stem cell homeostasis in the gut.** The panel on the left illustrates the influence of the microenvironment on colorectal cancer stem cell (CSC) plasticity and tumour survival. The upper right panel highlights the importance of R-spondin 3 (RSPO3) production by myofibroblasts for gastric stem cell activity. Elevated RSPO3 expression in response to *Helicobacter pylori* infection increases stem cell activity to combat the infection. The lower right panel shows Paneth cells supplying lactate for neighbouring stem cell metabolism in the intestinal crypt, and the cooperative effects of WNT–frizzled (WNT–FZD) and leucine-rich repeat-containing G-protein coupled receptor 5–RSPO (LGR5–RSPO) signalling on intestinal stem cell function.

In the intestine, Paneth cells comprise an important epithelial component of the stem cell niche[1]. This year, metabolic profiling revealed that Paneth cells display a glycolytic program, supplying lactate to fuel the largely oxidative phosphorylation program in the adjacent, mitochondria-rich ISCs[7]. Modulation of oxidative phosphorylation levels in isolated LGR5$^+$ intestinal cells directly influenced their stem cell activity *in vitro*, highlighting the importance of the distinct metabolic compartments within the ISC zone. Future studies should address whether the ISCs adapt their metabolic program in response to environmental changes such as nutrient availability or injury, or whether CSCs display similar metabolic profiles that can be therapeutically targeted.

The organoid culture system supports expansion of near-physiological gut epithelia for basic biology applications and preclinical screening. However, its clinical potential has been hampered by its reliance on poorly defined matrices that are incompatible with human use. To address this limitation, Gojorevski *et al.*[8] designed a synthetic hydrogel that closely mimics the native intestinal microenvironment. By engineering an intrinsic susceptibility to gradual hydrolytic degradation, they created a hydrogel that initially displays the rigidity needed to support ISC expansion, but later softens to provide the necessary conditions for differentiation into multicellular organoids. This breakthrough not only paves the way for the use of cultured intestinal stem cells or epithelia for clinical applications, but should also facilitate more accurate modelling of dynamic changes that occur during injury and disease.

Of particular clinical significance in 2017 was the discovery by two related studies that primary CRC can adapt to loss of the endogenous LGR5$^+$ CSC pool by reprogramming

differentiated cells to stem cells, implying that combination therapies targeting both the tumour bulk and either CSCs or the tumour microenvironment regulating CSC plasticity will be required to effectively treat primary CRC[9,10]. By contrast, liver metastases were unable to adapt to loss of their resident LGR5$^+$ CSCs, highlighting the influence of the local tumour microenvironment on cancer cell plasticity[10]. This latter observation also provides renewed hope that targeted CSC therapies might benefit patients with CRC and liver metastases.

As we continue to expand our knowledge of gastrointestinal stem cells, our ability to safely manipulate these cells for regenerative medicine applications will undoubtedly improve. The impressive advances made in 2017, driven by high-quality basic science, bring us ever closer to realizing the massive regenerative potential of gut stem cells in the clinic.

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HYPERTENSION IN 2017

Novel mechanisms of hypertension and vascular dysfunction

Ernesto L. Schiffrin

New findings in 2017 enhanced our understanding of the mechanisms that regulate blood pressure. Key studies provided insights into immune mechanisms, the role of the gut microbiota, the adverse effects of perivascular fat and inflammation on the vasculature, and the contribution of rare variants in renin–angiotensin–aldosterone system genes to salt sensitivity.

The year 2017 saw the publication of many basic and clinical studies in the field of hypertension as well as new American Heart Association and American College of Cardiology guidelines for the management of high blood pressure in adults[1]. Here, I focus on key studies that advanced understanding of basic mechanisms of blood pressure regulation and vascular dysfunction, including three studies that provide new insights into the salt sensitivity of blood pressure.

Research into immune mechanisms of hypertension has grown substantially in recent years and 2017 was no exception. As angiotensin II (ANGII) infusion increases blood pressure via a mechanism involving increased production of IL-17 from T helper 17 (T_H17) cells in mice[2], and salt induces T_H17 cells via a serum and glucocorticoid-regulated kinase 1 (SGK1)-dependent pathway[3], Norlander *et al.* evaluated whether SGK1 signalling in T cells contributes to salt-induced blood pressure elevation and end-organ damage[4]. They report that in mice, T cell-specific deletion of *Sgk1* blunted blood pressure elevation and abrogated endothelial dysfunction and renal injury in response to ANGII infusion. Deoxycorticosterone acetate–salt-induced blood pressure elevation and vascular inflammation were also attenuated in these mice. The basolateral $Na^+/K^+/2Cl^-$ cotransporter 1 (NKCC1; also known as solute carrier family 12 member 2) was upregulated in $CD4^+$ T cells cultured in T_H17-polarizing conditions and mediated a salt-induced increase in the expression of SGK1 and the IL-23 receptor (which has a role in T_H17 cell differentiation). Thus, T cell SGK1 and NKCC1 are novel mediators of the effects of salt on blood pressure and are potential targets for the treatment of hypertension.

Wilck *et al.* also reported a novel mechanism by which salt increases blood pressure and induces T_H17 cells[5]. They found that in mice, high salt intake results in changes in bacterial species in the gut, particularly depletion of *Lactobacillus murinus*. Moreover, treating mice with *L. murinus* prevented a salt-induced increase in T_H17 cell numbers, hypertension and the worsening of experimental autoimmune encephalomyelitis. In healthy men, a high-salt diet led to a reduction in intestinal *Lactobacillus* spp. and increases in T_H17 cell numbers and blood pressure. These results establish a link between salt intake and the gut microbiome, and between the gut microbiome and the immune system. The researchers propose that the gut microbiome might be a potential therapeutic target to counteract salt-sensitive conditions, including hypertension.

A role of another lymphocyte subset — γδT cells — in blood pressure elevation was demonstrated by Caillon *et al.*[6] These unconventional, innate-like T cells comprise only 1–4% of all lymphocytes in the circulation but are very abundant in some tissues such as the intestinal wall and the skin. In wild-type mice, ANGII infusion increased systolic blood pressure, increased splenic γδT cell numbers and activation, and decreased endothelial function. These effects on blood pressure and endothelial function were abrogated in mice that lacked γδT cells owing to genetic knockout of the δ-subunit or antibody-induced γδT cell depletion. ANGII-induced T cell activation in the spleen and perivascular adipose tissue was also blunted in *Tcrδ*$^{-/-}$ mice. In a cohort of people with or without coronary artery disease and/or hypertension, a multiple linear regression model showed similar, additive correlations between TCRγ constant region expression in blood, age and sex; the addition of coronary artery disease to the model did not improve these correlations. Thus, γδT cells might contribute to the development of hypertension and are a novel target for therapy.

The incidence of aortic aneurysms is increased in patients with hypertension and atherosclerosis compared with the general population. In 2017, increasing evidence for a role of perivascular fat and inflammation in vascular remodelling led to a study of the potential role of perivascular visceral adipose tissue (VAT) in aneurysm formation. Sakaue *et al.*[7] tested the hypothesis that genetic deletion of type 1a angiotensin II receptor (AGTR1A) in VAT could blunt the development of aortic aneurysms in apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice. They found that, compared with transplantation of VAT from *Apoe*$^{-/-}$ mice, transplantation of VAT from *Apoe*$^{-/-}$/*Agtr1a*$^{-/-}$ mice to around the abdominal aorta of *Apoe*$^{-/-}$ recipients reduced

Key advances

- Serum and glucocorticoid-regulated kinase 1 (SGK1) signalling in T cells contributes to salt-induced blood pressure elevation and end-organ damage via a mechanism involving upregulation of $Na^+/K^+/2Cl^-$ cotransporter 1 (NKCC1) and polarization to a T helper 17 (T_H17) cell phenotype[4]
- A high-salt diet alters the gut microbiome of humans and mice, resulting in depletion of *Lactobacillus* spp. and an increase in T_H17 cell numbers and blood pressure[5]
- In mice, γδT cells contribute to angiotensin II-induced hypertension and endothelial dysfunction and might also have a role in human hypertension and end-organ damage[6]
- AGTR1A and osteopontin mediate the polarization of macrophages to an inflammatory phenotype in perivascular fat, resulting in inflammation and potentially contributing to the formation of aortic aneurysms[7]
- Rare variants in seven renin–angiotensin–aldosterone system (RAAS)-related genes, including *APLN* and *RENBP*, are associated with salt sensitivity of blood pressure[8]

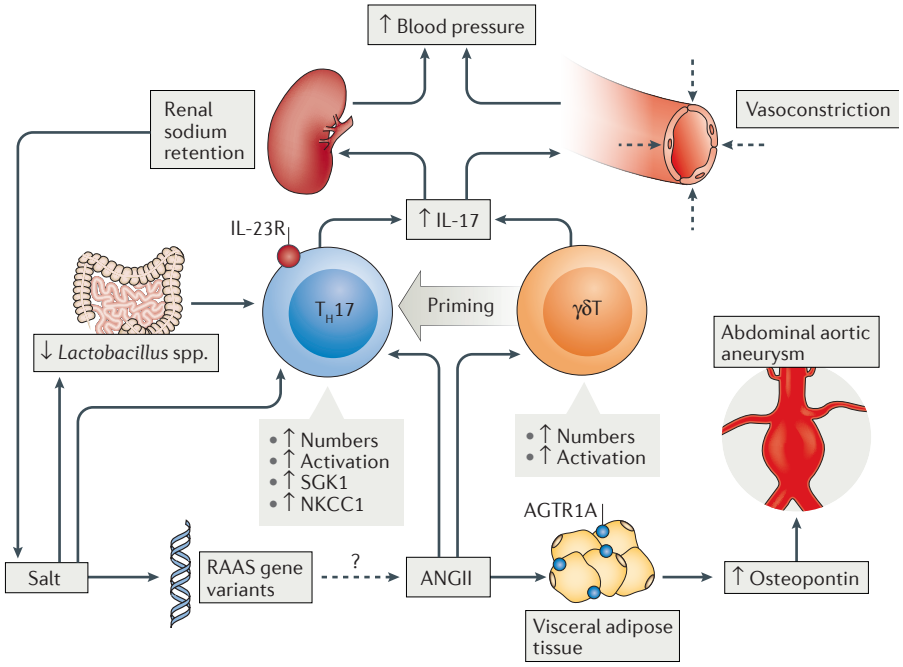

Figure 1 | **Novel mechanisms of hypertension and aortic aneurysm formation.** Salt induces activation of T helper 17 (T_H17) cells via a serum and glucocorticoid-regulated kinase 1 (SGK1)-dependent pathway that enhances the activity of $Na^+/K^+/2Cl^-$ cotransporter 1 (NKCC1). High salt intake also results in depletion of *Lactobacillus* spp. in the intestinal microbiome, leading to stimulation of T_H17 cells. Angiotensin II (ANGII) stimulates γδT cells, which in turn prime other immune cells, including T_H17 cells. Activated T_H17 and γδT cells produce IL-17, which stimulates renal sodium retention and vasoconstriction, resulting in increased blood pressure. Rare renin–angiotensin–aldosterone system (RAAS) gene variants increase the salt sensitivity of blood pressure, potentially via a mechanism involving ANGII. In perivascular visceral adipose tissue, binding of ANGII to type 1a angiotensin II receptor (AGTR1A) leads to increased production of pro-inflammatory osteopontin, which contributes to the formation and progression of abdominal aortic aneurysms.

the formation of aortic aneurysms, infiltration of macrophages and gelatinolytic activity in the abdominal aorta. In addition, AGTR1A activation polarized VAT macrophages to an inflammatory phenotype, and AGTR1A deficiency resulted in a reduction in the expression of pro-inflammatory osteopontin in VAT and in ANGII-induced osteopontin production by cultured adipose cells. Moreover, treatment with an osteopontin-neutralizing antibody reduced ANGII-induced macrophage migration. Consistent with these findings, the researchers showed that transplantation of VAT from osteopontin-deficient *Apoe*$^{-/-}$ mice was more effective in reducing formation of aortic aneurysms than was transplantation of *Apoe*$^{-/-}$ VAT. They conclude that VAT AGTR1A has a role in the formation of abdominal aortic aneurysms via a mechanism involving osteopontin. These findings could potentially prompt use of inhibitors of the renin–angiotensin system to prevent the development or progression of aortic aneurysms in at-risk patients, including those with hypertension.

Finally, a discussion of recent advances in the understanding of hypertension would not be complete without the inclusion of a genetic study. Genome-wide association studies (GWAS) and candidate gene studies have identified common genetic variants that influence the salt sensitivity of blood pressure, including numerous single-nucleotide polymorphisms in renin–angiotensin–aldosterone system (RAAS) genes[8]. However, these common variants explain only a small part of the heritability of blood pressure sensitivity to salt. In 2017, Kelly *et al.* conducted a resequencing study in which they evaluated the associations of rare variants of seven RAAS genes with blood pressure salt sensitivity in the 300 most salt-sensitive and 300 most salt-resistant participants of the GenSalt study[9]. The seven genes (*RENBP*, *ACE2*, *AGTR1*, *HSD11B1*, *HSD11B2*, *NR3C2* and *APLN*) were selected based on their potential roles in the regulation of blood pressure. The researchers found that individuals with rare variants in these genes had 1.5-fold greater odds of being salt sensitive than those without rare variants. In addition, the *APLN* gene was associated with salt sensitivity and rare *APLN* variants conferred 2.2-fold increased odds of salt sensitivity. Analyses of 50 common and low-frequency variants identified associations between single markers of the remaining six RAAS genes and salt-sensitive phenotypes. After adjustment for multiple testing, however, only the *RENBP* variant rs78377269 was associated with salt sensitivity. Each copy of the minor allele of this variant resulted in a 1.6 mmHg greater blood pressure response to increased dietary sodium and was associated with a doubling of the odds of salt sensitivity. This study provides the first evidence of a potential contribution of rare RAAS gene variants to the salt sensitivity of blood pressure.

In summary, key studies published during 2017 open new vistas into mechanisms of blood pressure elevation and aortic aneurysm formation that bring together salt, immunity, genetics, the RAAS and the vasculature (FIG. 1). These advances provide opportunities for the discovery of novel biomarkers and therapeutic targets for hypertension.

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RENAL METABOLISM IN 2017

Glycolytic adaptation and progression of kidney disease

Ton J. Rabelink and Peter Carmeliet

Studies of cellular energetics have revealed important roles of metabolic pathways in determining cell fate and response to injury. Insights from 2017 into the mechanisms underlying these pathways might identify therapeutic targets to minimize injury and promote repair.

Accumulating evidence has demonstrated a link between cell metabolism and cell fate decisions. In particular, glycolytic flux is now recognized as a key driver of self-renewal and cell proliferation and is critical to processes such as angiogenesis and tumour growth[1]. Glycolysis can be activated by energy deficits as well as through growth factor signalling (FIG. 1). In response to these signals, nutrient-sensing protein complexes, such as AMP-activated protein kinase (AMPK) and its target 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), increase the flow of the glycolytic end products into the tricarboxylic acid cycle to induce energy production within mitochondria[2]. This process is balanced by the use of glycolytic intermediates for macromolecular synthesis, redox homeostasis and epigenetic regulation. Mitochondrial function is key to the calibration between these catabolic and anabolic pathways. In the settings of diabetes mellitus and kidney disease, mitochondrial dysfunction and uncoupling from energy metabolism has been demonstrated. Several papers in 2017 highlighted the importance of glycolysis and mitochondrial function in development and function of the kidney and the effects of their dysregulation on the progression of kidney disease.

One interesting study from 2017 showed that glycolysis, and the associated capacity for cellular energy production, are pivotal, cell-intrinsic determinants of nephron progenitor fate; a high glycolytic flux was shown to support the self-renewal of progenitors, whereas inhibition of glycolysis stimulated their differentiation[3]. In the developing kidney, nephron progenitor cells (NPCs) reside in a niche that includes the cap mesenchyme, the ureteric tips and the surrounding stroma, where they have the capacity to self-renew and thus expand the progenitor cell pool or differentiate into epithelial structures[4]. In their study, Liu *et al.* demonstrated that young NPCs (at day 13 of embryogenesis) displayed higher glycolytic flux and higher mitochondrial respiratory capacity than older NPCs (present at birth). Inhibition of glycolysis stimulated NPC differentiation and nephrogenesis, depleting the niche of NPCs.

The finding that a similar metabolic switch might operate in the postnatal kidney to control cell proliferation and repair is notable. For example, glycolysis is activated in tubular epithelium upon acute kidney injury, as seen following ischaemic insults[5]. In line with the observations in NPCs, it is tempting to hypothesize that the activation of glycolysis in response to injury contributes to epithelial proliferation and regeneration of the tubular compartment. In the injured kidney, however, this glycolytic response occurred together with mitochondrial dysfunction, suggesting that it is not a reparative mechanism. Indeed, the number of mitochondria were greatly reduced following injury, and large autophagolysosomes were found in association with tubular atrophy and fibrosis[5]. A further study explored whether the transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) — which regulates metabolism and, in particular, mitochondrial biogenesis — can be targeted to overcome mitochondrial defects resulting from kidney injury[6]. The researchers reported decreased levels of PGC1α in the renal epithelium of biopsy samples from patients with chronic kidney disease in association with overexpression of Notch, a well-established profibrotic factor. Further studies showed that Notch directly downregulates PGC1α transcription. *In vitro*, overexpression of PGC1α in tubular epithelial cells restored mitochondrial content and the mitochondrial

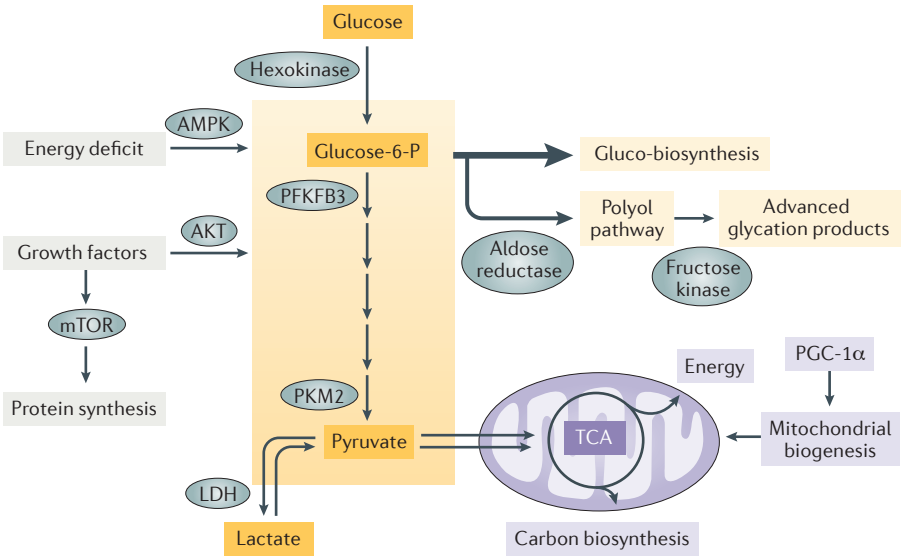

Figure 1 | **Glycolytic cell metabolism.** Cellular glucose is shuttled by hexokinase into the glycolytic pathway. Some of the glycolytic products are used for gluco-biosynthesis (for example, glycosoaminoglycan production and glycosylation processes) and for energy production. The key activating enzymes of glycolysis, such as 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), are regulated by cellular energy status (through AMP-activated kinase (AMPK) activity) or growth factor signalling (through AKT), which is also closely linked to mechanistic target of rapamycin (mTOR)-regulated protein synthesis. Generation of the final product of glycolysis, pyruvate, is governed by pyruvate kinase M2 (PKM2), which also stimulates mitochondrial function. The tricarboxylic acid (TCA) cycle calibrates cellular energy production with biomass production, using pyruvate (and lactate-derived pyruvate), glutamine and free fatty acids as an energy source. Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), like PKM2, indirectly stimulates mitochondrial biogenesis and function and increases respiratory capacity. In the presence of mitochondrial dysfunction, glycolytic intermediates accumulate and shuttle into the polyol pathway, where they are metabolized into fructose and then into toxic advanced glycation end products. LDH, lactate dehydrogenase; Glucose-6-P, glucose-6-phosphate.

Key advances

- Increased glycolysis and mitochondrial respiratory capacity stimulate cell proliferation and self-renewal of progenitor populations[3]
- Acute kidney injury and diabetic nephropathy are characterized by mitochondrial dysfunction and the accumulation of glycolytic intermediate products, which can be metabolized into toxic end products[5,9]
- Coupling of glycolytic flux to mitochondrial respiratory function is emerging as a potential therapeutic approach to reduce ischaemic and diabetic kidney injury[6,8,10]

respiratory defect induced by Notch signalling. These findings were translated to an *in vivo* mouse model in which overexpression of PGC1α ameliorated renal fibrosis induced by tubular epithelial overexpression of Notch. Together, these data emphasize the relevance of mitochondrial function in coupling energy metabolism to cell fate and repair capacity.

The combination of increased glycolysis and impaired mitochondrial function results in an accumulation of glycolytic intermediate products that overflow into the polyol pathway, where they are metabolized to fructose[7]. Fructose is subsequently phosphorylated by fructokinase, leading to the formation of uric acid and advanced glycation end products. The pathophysiologic relevance of this glycolytic overflow system in kidney injury and repair was highlighted by an elegant study that showed that fructokinase haploinsufficiency protects mice from ischaemic kidney injury[8]. Compared to wild-type mice with ischaemic kidney injury, fructokinase-deficient mice had reduced ATP depletion, lower renal uric acid levels, lower markers of oxidative stress and reduced kidney injury.

The coupling of glycolytic flux to mitochondrial respiration is also pertinent in diabetes mellitus. Similarly to acute kidney injury, glycolysis is also increased in diabetes mellitus and seems to be uncoupled from mitochondrial respiration. For example, a 2016 study showed that urinary markers of increased renal glycolysis and fatty acid oxidation, together with the presence of altered mitochondrial proteins, could predict the occurrence of nephropathy in patients with diabetes mellitus[9]. Increased glycolytic flux can itself, however, prevent the build-up of toxic glucose intermediates that occur in the diabetic environment, as long as mitochondrial respiratory capacity is sufficient to use the pyruvate that is produced and to keep glycolytic flux going. Arguably, the most exciting paper of 2017 in this respect is that by Qi *et al.*, who performed a proteomics analysis on glomeruli of patients with extreme duration of diabetes (>50 years) with and without diabetic nephropathy[10]. Patients without diabetic nephropathy had elevated levels of enzymes involved in regulating glycolysis and mitochondrial respiration. In particular, elevated pyruvate kinase M2 (PKM2) was identified as a protective factor against the development of diabetic nephropathy — a finding that is of great interest given that PKM2 is usually suppressed in patients with diabetes mellitus and is known to couple glycolysis to mitochondrial respiration. The investigators demonstrated that diabetic mice with podocyte-specific deletion of PKM2 had more severe albuminuria and glomerular pathology than diabetic control mice. Furthermore, pharmacological activation of PKM2 reduced the hyperglycaemia-induced elevation in toxic glucose metabolites and mitochondrial dysfunction, partially by increasing glycolytic flux and levels of PGC1α.

Collectively, these papers provide a solid scientific basis from which to further explore the role of cellular energetic adaptations, such as the interplay between glycolysis and mitochondrial respiration, in kidney injury. Of note, metabolic processes vary widely from one cell type to another — a point that is of particular importance in the kidney, as demonstrated by the reliance of tubular epithelial cells on mitochondrial respiration and the endothelium on glycolysis. Thus, further research into the effects of metabolism, mitochondrial function interventions in the kidney and the development of therapeutic interventions, will likely require greater insights into the metabolic adaptation mechanisms of specific cell types.

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IMMUNE-MEDIATED KIDNEY DISEASE IN 2017

Progress in mechanisms and therapy for immunological kidney disease

Stephen R. Holdsworth and A. Richard Kitching

In 2017, progress was made in several aspects of immune-mediated kidney disease. Mechanistic studies provided new insights into the underlying signals that confer risk to, or protection from, immune pathways, whereas new approaches to the treatment of immunological kidney disease will hopefully translate into a move away from the use of toxic corticosteroids.

Progress in understanding the pathogenesis of immune-mediated glomerular disease and refining its treatment continued over the past year, with excellent contributions to this field in 2017. In the field of molecular translational research, two contributions established new ways of understanding disease pathogenesis and approaches for better disease management. In terms of therapeutics, we highlight clinical trials centred on the vexed question of the role

Table 1 | **Clinical trials of corticosteroids and alternative therapies in immune-mediated kidney disease**

	TESTING	NEFIGAN	CLEAR
Disease	IgA nephropathy	IgA nephropathy	ANCA-associated glomerulonephritis
Design (duration; number of patients)	• Phase III, double blind (36 months; 262)	• Phase IIb ,double blind (9 months; 150)	• Phase II, double blind, noninferiority stepwise (12 weeks; 67)
Intervention	• Oral methylprednisolone vs placebo • RAS blockade included in both arms	• TRF-budesonide vs placebo • RAS blockade included in both arms	• Avacopan ± corticosteroids vs corticosteroids alone • Included cyclophosphamide or rituximab
Outcome	Early termination of trial	Improvement in proteinuria with TRF-budesonide vs placebo	• Similar improvement in BVAS; more rapid improvement in albuminuria • Fewer steroid-type AEs with avacopan
Caveats/ongoing trials	• Trial terminated early owing to sepsis-related death from corticosteroid toxicity. May otherwise have shown efficacy • TESTING Low Dose Study in progress (NCT01560052)	• Some systemic corticosteroid-type AEs in treatment groups • No systemic oral corticosteroid comparator • Phase III trial planned for 2018	• Small numbers and short-term study • Sequential enrolment design • Relatively preserved mean eGFR at randomization • Phase III trial in progress (NCT02994927)

AEs, adverse effects; ANCA, anti-neutrophil cytoplasmic antibody; BVAS, Birmingham Vasculitis Activity Score; eGFR, estimated glomerular filtration rate; RAS, renin–angiotensin system; TRF, targeted-release formulation.

of corticosteroid treatment for proliferative glomerulonephritis and emerging therapies that may reduce use of these agents.

One study[1] defined the fundamental mechanism by which the human leukocyte antigen (HLA) system mediates the risk of, and protection from, immune renal disease. The HLA genotype is the most prominent genetic risk factor in most autoimmune diseases. Although epidemiological studies have linked the HLA genotype to autoimmune disease, the mechanistic basis of this HLA-mediated risk is not known. Using anti-glomerular basement membrane (GBM) glomerulonephritis as a prototypic autoimmune disease, we identified how HLA-DR1 mitigates the HLA-DR15-mediated risk of disease. Although rare, anti-GBM disease has well documented HLA associations, with HLA-DR15 being the dominant HLA type that confers risk[2]. Moreover, the anti-GBM disease-causing autoantigen α3(IV)NC1 and its immunodominant T cell epitope $\alpha3_{135–145}$ are well defined and are similar in human and mouse. Using human *in vitro* models of autoimmunity and humanized HLA-transgenic mouse studies, we established that in the context of the dominantly protective HLA-DR1 allomorph, $\alpha3_{135–145}$-specific regulatory T (T_{reg}) cells prevent potentially damaging autoreactive $CD4^+$ T helper cells from emerging. When present, these T helper cells stimulate anti-GBM antibody production and act as effector cells in the kidney, causing damage. Structural studies revealed differences in the way that HLA-DR15 and HLA-DR1 present $\alpha3_{135–145}$ to T cells, leading to different T cell populations being selected. Thus, despite presentation of the same epitope, expression of HLA-DR1 generates a high proportion of T_{reg} cells that maintain tolerance in the periphery, whereas expression of HLA-DR15 leads to the generation of potentially damaging T helper cells. In anti-GBM disease and potentially in other autoimmune diseases, protective HLA types provide an important layer of tolerance through the actions of antigen-specific T_{reg} cells in the periphery. In addition to establishing a mechanism for the role of HLA genotypes in disease, this study demonstrates the potential therapeutic relevance of antigen-specific T_{reg} cells, highlights the link between T cell reactivity and the presence of autoantibodies, and validates the value of epitope discovery. It also establishes a platform with which to address the mechanisms by which immune tolerance is generated and maintained.

The factors that initiate anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) are ill defined. Major advances include definition of the target autoantigens, proteinase 3 (PR3) and myeloperoxidase (MPO) and their dysregulated expression during active disease[3]. A new longitudinal cohort study suggests that the aberrant overexpression of these autoantigens in active AAV is epigenetically determined[4]. Jones *et al.* found that expression of DNA methyl transferase 1 (*DNMT1*), which encodes a protein that is important in DNA methylation, was reduced at loci encompassing the autoantigen-encoding genes *MPO* and *PRTN3* in patients with active disease. Inhibition of DNA methylation at these sites was relatively specific, as the genome-wide range of methylation in patients with active disease was similar to that of normal, healthy controls. The extent of DNA methylation at these sites correlated negatively with *MPO* and *PRTN3* expression and increased significantly in patients in remission, suggesting that epigenetic mechanisms mediate aberrant autoantigen expression and disease induction. Although increased methylation of *PRTN3* was observed in patients with MPO and PR3 autoantigens, restitution of methylation at *MPO* loci was observed only in patients with MPO-AAV and not in those with PR3-AAV. These findings highlight the presence of genetic differences between patients with autoimmunity to MPO and PR3 autoantigens and are in accordance with data from genome-wide association studies that indicate MPO-AAV and PR3-AAV are two related but genetically different diseases[5]. The clinical relevance of DNA methylation was highlighted by the finding that the average time to relapse for patients with increased methylation of autoantigen loci was longer than

Key advances

- HLA associations with disease susceptibility in anti-glomerular basement membrane disease are explained by differences in the binding of the immunodominant peptide to different HLA types[1]
- DNA methylation of autoantigens, inducing anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, correlates with and may predict disease activity and remission[4]
- The adverse effects of high dose corticosteroids outweigh the potential therapeutic benefits in IgA nephropathy[6]
- Selective targeting of gut immune tissues with targeted-release formulation (TRF)-budesonide offers effective treatment of IgA nephropathy with the potential to markedly reduce systemic steroid exposure[7]
- Promising outcomes with use of a small molecule C5a receptor inhibitor confirms an important role for complement in ANCA-associated glomerulonephritis and is as effective as prednisolone in inducing disease remission[9]

for those with decreased methylation and that demethylation at the *PRTN3* promoter region was the strongest predictor of relapse. These studies confirm the importance of epigenetic effects on disease activation through changes in autoantigen expression — a finding that has relevance not only for our understanding of the pathogenesis of AAV but also for identifying potential biomarkers of disease activity.

Corticosteroids are widely used for the treatment of immune-mediated kidney diseases but have considerable adverse effects, as illustrated by the premature termination of the phase III TESTING trial of high-dose steroids in patients with IgA nephropathy (IgAN)[6] (TABLE 1). Some progress has been made in the identification of effective but less toxic alternative therapies for patients with kidney disease. On the basis of evidence that IgAN is directed by gut mucosal autoimmunity, a new study assessed the efficacy of a targeted-release formulation (TRF) of budesonide, which is designed to deliver the drug to the terminal ileum and releases <10% of the dose into the circulation. In the phase IIb NEFIGAN randomized controlled trial, Fellstrom *et al.*[7] reported a 24.4% reduction in proteinuria from baseline amongst patients on treatment compared with a 2.7% increase in proteinuria amongst those on placebo. Despite the promise of fewer adverse events, more study participants in the TRF-budesonide arm than in the placebo arm withdrew from the trial owing to adverse effects. Future phase III studies involving a conventional corticosteroid treatment arm are needed to assess the effectiveness of this formulation in patients with IgAN.

Despite the proliferation of targeted therapies for many inflammatory and autoimmune diseases, few thus far have been shown to be effective in immune-mediated kidney diseases[8]. The phase II, randomized, controlled CLEAR study[9] provides evidence that strategies to modulate the complement system might be effective in patients with AAV and renal involvement and could potentially replace or minimize corticosteroid induction therapy. Murine models have demonstrated a pathogenic role for the alternative pathway of complement and a critical role for C5a and the C5a receptor (C5aR) in particular[10]. Avacopan (CCX168) is an oral C5aR antagonist that was shown to alleviate ANCA-induced glomerular injury in mice expressing human C5aR. In the CLEAR trial, 80% of patients with acute vasculitis achieved the primary efficacy measure of a 50% reduction in Birmingham Vasculitis Activity Score by week 12, with avacopan and avacopan-plus-low-dose prednisolone treatment arms showing noninferiority to standard therapy. Patients on avacopan showed greater early improvement in albuminuria than those on standard treatment, and this difference remained evident after 12 weeks. Improvement in urinary levels of the chemokine MCP1 was greater in avacopan-treated groups than in patients who received standard therapy, suggesting that the anti-inflammatory effects of C5aR blockade might be greater than those of prednisolone. The incidence of worsening vasculitis and of serious adverse events was similar across all groups, but fewer adverse events related to corticosteroid therapy were reported in the avacopan groups. Although encouraging, this small phase II trial was of relatively short duration, and a larger phase III study (ADVOCATE) is underway. Together, these studies have uncovered new mechanisms of immune-mediated kidney disease and identified new pathways to target. Follow-up on these findings have the potential to change the outlook for affected patients.

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Competing interests statement
The authors declare no competing interests.

DIABETIC KIDNEY DISEASE IN 2017

A new era in therapeutics for diabetic kidney disease

Christoph Wanner

2017 saw the emergence of a new era in renoprotective medicine for diabetic kidney disease with reports of promising renal outcomes with the sodium–glucose cotransporter 2 (SGLT2) inhibitors empagliflozin and canagliflozin from follow-up analyses of the EMPA-REG OUTCOME trial and the CANVAS Program, respectively, and with use of the glucagon-like peptide 1 (GLP1) agonist liraglutide in the LEADER trial.

Since the publication 16 years ago of the IDNT and RENAAL trials, which showed beneficial effects of the angiotensin II-receptor blockers irbesartan and losartan, respectively in patients with type 2 diabetes mellitus (T2DM) and kidney disease, no new medications have been approved for the treatment of diabetic kidney disease[1,2]. Within the past year, however, two classes of antidiabetic medications, the sodium–glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide 1 (GLP1) analogues, have shown renoprotective potential[3–5], offering new hope for patients with diabetic kidney disease.

SGLT2 inhibitors work in an insulin-independent manner by promoting urinary glucose excretion and lowering blood pressure and body weight while avoiding hypoglycaemia. Concomitant inhibition of sodium absorption in the proximal tubule causes initial natriuresis, which leads to osmotic diuresis with an attendant reduction in plasma volume. Sodium sensing in the

macula densa activates tubuloglomerular feedback and adenosine-mediated dilation of the afferent renal arterioles. The reduction in (single-nephron) hyperfiltration and decrease in glomerular pressure is associated with a decline in glomerular filtration and albuminuria[6] (FIG. 1).

Primary analyses of the EMPA-REG OUTCOME trial of the SGLT2 inhibitor empagliflozin in patients with T2DM were published in 2016 and showed that patients on empagliflozin had a lower risk of incident or worsening nephropathy, doubling of serum creatinine level and initiation of renal replacement therapy (RRT) than those on placebo[3]. Two further analyses of the EMPA-REG OUTCOME trial, published in 2017, have focused on the effects of empagliflozin on albumininuria and other outcomes in patients with T2DM and established cardiovascular and chronic kidney disease (CKD). Of the 7,020 patients randomized in the EMPA-REG OUTCOME trial, 2,250 had prevalent kidney disease at baseline (defined as an estimated glomerular filtration rate (eGFR) 30–60 ml/min/1.73 m^2 and/or macroalbuminuria), of whom 84% were on renin–angiotensin system (RAS) blockers. Analyses from 2017 showed that empagliflozin reduced the risk of cardiovascular death in patients with prevalent CKD by 29% compared with placebo (HR 0.71; 95% CI 0.52–0.98) and the risk of all-cause mortality by 24% (HR 0.76; 95% CI 0.59–0.99)[7]. Effects of empagliflozin on these outcomes were consistent across categories of eGFR and urine albumin-to-creatinine ratio (UACR) at baseline. The risk reduction and decline in the progression of diabetic kidney disease was also prominent in patients with prevalent CKD[7]. Empagliflozin led to significant reductions in UACR from as early as week 12; this reduction in UACR was sustained for at least 3 years regardless of baseline albuminuria status and on top of the effects of RAS inhibition. The effect of empagliflozin on UACR seemed to be, in large part, independent of its effects on glycaemic control, suggesting a persistent renal haemodynamic effect of empagliflozin, which might confer short-term and long-term renal effects on UACR when used in addition to current standard of care[8].

Outcomes of a new trial published in 2017 — the CANVAS Program — reported benefits of a second SGLT2 inhibitor, canagliflozin, on renal outcomes among patients with T2DM at high cardiovascular risk[4]. Compared to placebo, canagliflozin reduced progression of albuminuria (HR 0.73; 95% CI 0.67–0.79) and the composite outcome of a sustained 40% reduction in eGFR, initiation of RRT or death from renal causes (HR 0.60; 95% CI 0.47–0.77). Thus, available data suggest that empagliflozin and canagliflozin reduce albuminuria in patients with either microalbumiuria or macroalbuminuria by about 25–35% (placebo corrected) from baseline. Although the composite renal end point cannot be directly compared between the CANVAS Program and EMPA-REG OUTCOME trial, the overall renal risk reduction seems to be similar in both trials[3,4].

Further advances in the treatment of diabetic kidney disease came with the 2017 publication of the LEADER trial of the GLP1 analogue liraglutide. GLP1 analogues improve glycaemic control through various mechanisms, including enhancement of insulin synthesis and secretion, β-cell proliferation and neogenesis. The precise mechanisms by which this agent provides renal protection beyond intensified glucose control are as yet unexplained, but are likely multifactorial, and might involve effects on inflammation and oxidative stress, rather than haemodynamic effects. Interestingly, liraglutide induces a very early and short-term initial increase in GFR that is probably mediated by nitric oxide — an effect that requires further explanation[5] (FIG. 1).

In the LEADER trial, liraglutide was associated with a lower risk of the prespecified secondary renal outcomes (a composite of new-onset persistent macroalbuminuria,

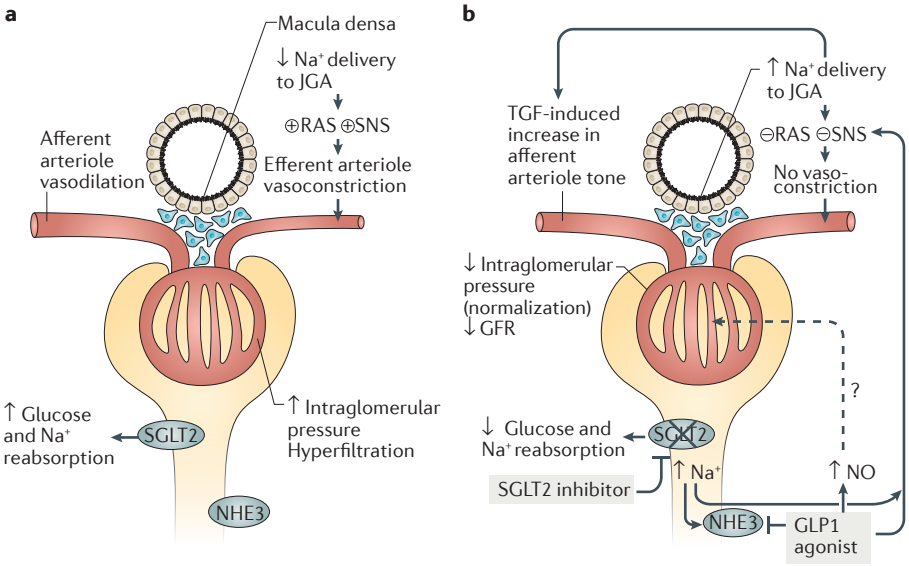

Figure 1 | **Effects of SGLT2 inhibition and GLP1 agonism on renal haemodynamics in diabetes mellitus. a** | Haemodynamic alterations induced by hyperglycaemia and diabetes mellitus. Glucose and sodium reabsorption in the proximal tubule is maximally activated and reduced amounts of sodium are delivered to the juxtaglomerular apparatus. The macula densa, via tubuloglomerular feedfack (TGF), induces vasodilation of the renal afferent arteriole, leading to an increase in intraglomerular pressure and hyperfiltration. **b** | Sodium–glucose cotransporter 2 (SGLT2) inhibition induces an increase in afferent arteriole tone and a subsequent decrease in intraglomerular pressure with a transient lowering of glomerular filtration rate (GFR). The integrated effect of glucagon-like peptide 1 (GLP1) agonism on renal haemodynamics seems to be the result of nitric oxide (NO)-induced vasodilation and inhibition of glomerular hyperfiltration. JGA, juxtaglomerular apparatus; NHE3, sodium–hydrogen exchanger isoform 3; RAS, renin–angiotensin system; SNS, sympathetic nervous system.

Key advances

- The CANVAS Program and follow-up analyses from the EMPA-REG OUTCOME trial have reported beneficial effects of sodium–glucose cotransporter 2 (SGLT2) inhibitors on renal outcomes, including albuminuria, and on 'harder' end points such as doubling of creatinine level, renal replacement therapy or renal death[4,7,8]
- The glucagon-like peptide 1 (GLP1) agonist liraglutide resulted in lower rates of the development and progression of diabetic kidney disease in the LEADER trial[5]
- In persons with pre-existing kidney disease and estimated glomerular filtration rate 30–60 ml/min/1.73 m^2 and/or macroalbuminuria, empagliflozin significantly reduced the risks of time to cardiovascular death, all-cause mortality, hospitalization for heart failure and all-cause hospitalization[7]

doubling of serum creatinine level, end-stage renal disease or death due to renal disease) than placebo (HR 0.78; 95% CI 0.67–0.92). This result was driven primarily by a reduction in new onset of persistent macroalbuminuria (HR 0.74; 95% CI 0.60–0.91) and not by effects on doubling of serum creatinine or initiation of RRT[5].

Of note, all renal outcomes in the CANVAS Program, the EMPA-REG OUTCOME trial and the LEADER trial were assessed as secondary end points. Although based on prespecified hypotheses, these findings are not viewed as robust as primary outcome data, and regulatory agencies have requested that dedicated renal outcome studies are performed for these agents to be approved for diabetic kidney disease.

Also of note are safety considerations associated with use of these agents. The increased risk of genital infections and ketoacidosis associated with use of SGLT2 inhibitors can likely be mitigated through diligent personal hygiene measures and by avoiding prolonged fasting in insulin-dependent patients. Lower limb amputation seems to be a particular concern for canagliflozin, for as yet unknown reasons[4]. GLP1 agonists are generally well tolerated and nausea is the most common adverse effect with incidence rates varying from 25–60%.

The renal findings from clinical trials of empagliflozin, canagliflozin and liraglutide are therefore encouraging and seem to herald a new era in renoprotective medicine. Further planned studies will specifically assess the efficacy of empagliflozin, canagliflozin and a third SGLT2 inhibitor, dapagliflozin, on renal outcomes and will also assess their efficacy in patients with non-diabetic CKD to establish whether the renal protective effects of these agents can be extended to all forms of kidney disease. Studies with further GLP1 agonists, albiglutide and dulaglutide, as well as oral forms of GLP1-analogues are also under way. So far, however, the results from available studies are encouraging and suggest that physicians might wish to consider the use of SGLT2 inhibitors and/or GLP1 analogues for the treatment of diabetic kidney disease and albuminuria in patients with declining renal function but without prior cardiovascular events, rather than postponing such treatment until the patient has survived a myocardial infarction.

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GENETICS OF KIDNEY DISEASES IN 2017

Unveiling the genetic architecture of kidney disease

Olivier Devuyst

Technical advances in genome sequencing and association studies have yielded critical insights into the genetic architecture of kidney diseases. Here, I summarize four key studies from 2017 that deciphered the genetic basis of known and novel diseases and provided insights into the mechanisms of glomerular, developmental defects and manifestations of kidney disorders.

Advances in genetic testing have provided major breakthroughs in our understanding of kidney diseases[1,2]. In 2017, four studies in particular integrated genetic evidence and multidisciplinary investigations to provide insights into the genetic heterogeneity, mechanisms and manifestations of kidney diseases.

Causative mutations can now be detected in ~30% of individuals with early-onset steroid-resistant nephrotic syndrome (SRNS)[3]. Further deciphering the genetic aetiology of SRNS remains a challenge. Using whole exome sequencing, Lovric *et al.* identified recessive mutations in *SGPL1*, which encodes sphingosine-1-phosphate (S1P) lyase, in seven families with a newly described form of syndromic SRNS[4]. SGPL1 is an endoplasmic reticulum (ER) enzyme that drives the final step of the sphingolipid breakdown pathway by degrading S1P, a sphingolipid metabolite that regulates complex processes (FIG. 1a). The mutations resulted in reduced or absent SGPL1 protein and/or enzyme activity, subcellular mislocalization of SGPL1 and altered ceramide composition of patient fibroblast-conditioned medium. *Sgpl1*$^{-/-}$ mice had proteinuria, acanthosis and immunodeficiency, with podocyte foot process effacement, absence of slit diaphragms and reduced numbers of mesangial cells. Knockdown of *Sgpl1* in rat mesangial cells inhibited cell migration, which was partially rescued by a S1P receptor antagonist. The role of SGPL1 was further verified in *Drosophila Sply* mutants that lack SGPL1: these flies

Key advances

- Recessive mutations in *SGPL1* cause dysregulation of sphingolipid catabolism and are associated with a syndromic from of steroid-resistant nephrotic syndrome[4,5]
- A recurrent deletion at the 22q11.2 locus drives kidney defects in DiGeorge syndrome and in congenital kidney and urinary tract anomalies; haploinsufficiency of *CRKL* is probably involved[6]
- The absence of positive family history, which poses a significant diagnostic challenge for autosomal dominant polycystic kidney disease, may be due to *de novo* disease, germline or somatic mosaicism, or mild disease from hypomorphic mutations in *PKD1* or *PKD2* (REF. 8)
- Unsuspected genomic disorders might impair both kidney and neurocognitive development in children; early identification of such genomic disorders might provide an opportunity for specific interventions[10]

demonstrated altered sphingolipid metabolism and defective nephrocyte function that could be rescued by wild-type *Sply*. Together, these results indicate that recessive mutations in *SGPL1* that cause dysregulation of sphingolipid catabolism are associated with a syndromic form of SRNS. The variable organ involvement might be explained by residual levels of SGPL1 activity or additional factors involved in S1P metabolism. Additional, homozygous mutations in *SGPL1* were also associated with SRNS and primary adrenal insufficiency, further emphasizing the impact of altered sphingolipid metabolism[5].

With an estimated prevalence of 1:2,000–4,000 live births, DiGeorge syndrome is the most common microdeletion syndrome. The syndrome is usually sporadic, resulting from a *de novo* deletion of a region of chromosome 22 (22q11.2), and is characterized by cardiac malformations, hypoparathyroidism with hypocalcaemia, and thymic hypoplasia that causes an immune deficit, short stature and neurodevelopmental defects; congenital kidney and urinary tract anomalies (CAKUT) occur in about 30% of cases. Haploinsufficiency of *TBX1*, which maps to 22q11.2, is thought to be responsible for the main features. However, *TBX1* is not expressed in the embryonic kidney and the identity of the gene(s) responsible for the kidney defects was unknown. Using a genome-wide search for rare copy-number variations (CNVs) in cohorts of patients with CAKUT, Lopez-Rivera *et al.*[6] identified heterozygous deletions of 22q11.2 in 12 of 1,093 (1.1%) patients and three of 22,094 (0.013%) controls (OR 81.5; $P = 4.5 \times 10^{-14}$) and defined a 370 kb region containing nine genes as the main driver of the kidney defects. Knockdown of *snap29*, *aifm3* and *crkl* resulted in comparable renal defects in zebrafish; loss of *crkl* alone — a gene that encodes an adaptor protein thought to regulate transcription factor signalling during kidney development (FIG. 1b) — was sufficient to induce the phenotype. The major role of *CRKL* in kidney development was supported by the discovery of deleterious *CRKL* variants in 1% of patients with sporadic congenital renal agenesis or hypodysplasia and by the developmental kidney and urinary tract defects secondary to inactivation of *Crkl* in mice. These studies reveal that a 370 kb deletion at 22q11.2 is the driver of developmental kidney defects in DiGeorge syndrome and sporadic CAKUT, with haploinsufficiency in *CRKL* being the main genetic factor involved.

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy, with a prevalence of ~1:500–1,000 living births. Mutations in *PKD1* and *PKD2* account for 85% and 15% of the affected families, respectively. *PKD1* and *PKD2* encode polycystin 1 and polycystin 2, respectively. A third gene, *GANAB*, has been identified in a few families presenting with a mild form of ADPKD and liver cystic disease of variable severity. *GANAB* encodes the α-subunit of glucosidase II, an enzyme that is required for the maturation and proper surface localization of polycystin 1 and polycystin 2 (REF. 7). An absence of apparent positive family history poses a diagnostic challenge in 10–25% of patients with suspected ADPKD. To define the genetic basis of disease in patients without an apparent family history of ADPKD, Iliuta *et al.* reviewed 210 affected patients who underwent comprehensive biological, imaging and genetic testing[8]. 58 (28%) did not have an apparent family history of ADPKD, 32 (15%) had *de novo* disease, 22 (11%) had indeterminate family history and four (2%) had positive family history in retrospect. Mutations in *PKD1* or *PKD2* were identified in 55% of patients with *de novo* disease and indeterminate family history compared with the 95% detection rate in patients with positive family history. Imaging review revealed atypical (asymmetric, unilateral or focal) PKD in 26% of patients with apparently negative family history, compared with 13% in patients with positive family history. Among patients with putative *de novo* ADPKD, half (16 of 32) had an identifiable *PKD1* or *PKD2* mutation; the *de novo* nature of disease was confirmed in eight of these 16 patients. Two families with germline mosaicism and one with somatic mosaicism were detected among the mutation-negative patients. Somatic mosaicism was also suspected in additional, mutation-negative patients with asymmetric PKD and indeterminate family history. Three of four patients with a positive family history in

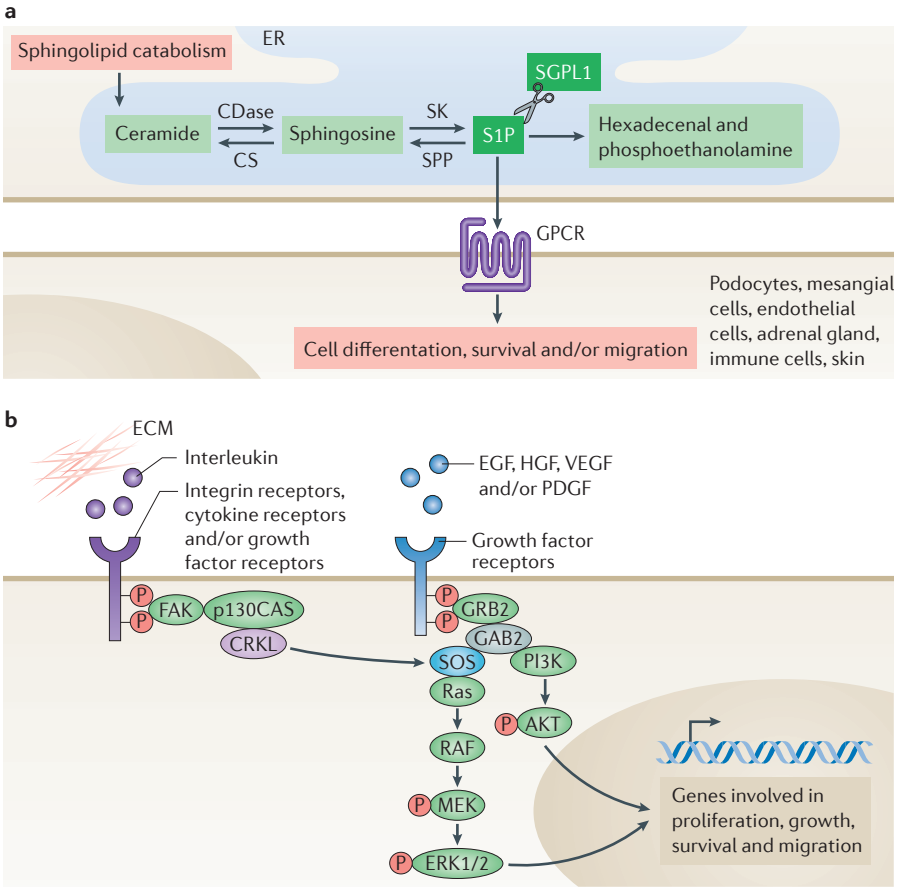

Figure 1 | **Novel pathways involved in congenital kidney disorders. a** | Role of SGPL1 in the sphingolipid catabolic pathway. SGLP1 is an endoplasmic reticulum (ER) enzyme that irreversibly cleaves the lipid-signalling S1P, which exerts multiple effects in different cell types and is mediated by the activation of a family of G protein-coupled receptors (GPCRs). **b** | Pathways associated with the adaptor protein CRKL, which facilitates signal transduction events downstream of receptor tyrosine kinases. CRKL is activated by various types of cell surface receptors, including growth factor receptors, cytokine receptors and integrin receptors, upon binding to extracellular matrix (ECM). The adaptor proteins CRKL and GAB2 lead to activation of MAPK and PI3K–AKT pathways. CDase, ceramidase; CS, ceramide synthase.

retrospect were found to have non-truncating *PKD1* mutations, classically associated with milder disease. After exclusion of patients with asymmetric PKD and those suspected or proven to harbour somatic mosaicism, 8.6% of patients were genetically unresolved. Thus, although the cause of disease can be identified in most patients with ADPKD, it remains unsolved in a small subset. Resequencing of *PKD1* and *PKD2* in different cell types might facilitate the diagnosis of mosaicism. Other causes may be atypical *PKD1* or *PKD2* mutations or mutations in other genes involved in polycystin processing.

20–40% of children with chronic kidney disease (CKD) present with impaired neurocognitive function, with high rates of depressive and anxiety manifestations. Beyond CKD, the involvement of genomic disorders (GDs) in neurocognitive impairment is an area of interest. Approximately 15% of children with intellectual disability or developmental delay carry a pathogenic CNV. Similarly, large CNVs are detected in ~15% of paediatric patients with renal hypoplasia or dysplasia and in 7% of the 419 participants of the CKD in Children (CKiD) Study[9]. A new study of neurocognitive performance in CKiD Study participants with known or likely pathogenic GDs demonstrated that compared with non-carriers ($n = 389$), children with GDs ($n = 31$) scored significantly poorer on neurocognitive tests[10]. Importantly, maternal education was inversely associated with severity of neurocognitive impairment in children with GDs, indicating potential modification by genetic and/or environmental factors. Thus, in a subset of paediatric patients with CKD, impaired neurocognitive function might be linked to GDs that affect both kidney and neurocognitive development. If confirmed in other cohorts, these findings argue for the early identification of GDs in paediatric patients with CKD to potentially enable early interventions to mitigate neurocognitive complications.

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Competing interests statement
The author declares no competing interests.

MOTOR NEURON DISEASE IN 2017

Progress towards therapy in motor neuron disease

Matthew C. Kiernan

In 2017, dramatic advances have been made in the treatment of motor neuron diseases. New therapies have been approved for spinal muscular atrophy and amyotrophic lateral sclerosis, and a host of other therapies that are currently under development are showing promising results.

Neurodegenerative disease represents the foremost public health challenge of our time; it exerts a devastating effect on quality of life and imposes a major burden on health-care systems. Among neurodegenerative conditions, motor neuron disorders such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are the most rapidly fatal, with progression from symptom onset to death typically taking place within 2 years[1]. Motor neurodegenerative disorders demonstrate a challenging and progressive disease course, with rapidly changing care needs and symptoms, including progressive weakness, disability, respiratory failure and early death. Past decades of research have provided extensive insights into the pathophysiology of neurodegenerative disease, but, to date, successful treatment options and appropriate assessment measures have remained limited. However, considerable progress has emerged over the past 12 months, with an unprecedented number of treatments now ready to be introduced to clinical practice and many more therapies in clinical trial development, which hold yet further promise (FIG. 1).

The most dramatic therapeutic advance for motor neuron disorders relates to clinical trials in SMA. The most frequent form of SMA is linked to genetic mutation of the survival motor neuron 1 gene (*SMN1*), which leads to SMN protein deficiency. Nusinersen (IONIS-SMN_{RX})is an antisense oligonucleotide therapy designed to increase functional SMN protein by modifying the splicing of *SMN2* (REF. 2). Following initial promise during phase I and II studies that confirmed safety and a minimal side effect profile even despite intrathecal administration, phase III trials in SMA type I (ENDEAR) and SMA type II (CHERISH) achieved their primary end points, with evidence of improvement in achievement of motor milestones[2]. Anyone who has read the trial data and witnessed accompanying patient videos of children surviving and maintaining motor milestones — often without evidence of reaching a plateau in their improvement — could not have failed to be impressed. Moving forward, the potential benefit of treatment in presymptomatic individuals is currently being evaluated, with results expected to determine the ideal therapeutic window for intervention.

In further therapeutic developments for motor neuron diseases, the FDA approved the use of a new treatment in ALS. Edaravone was originally developed as a free-radical scavenger to treat stroke patients in Japan. Initial studies undertaken in stroke showed that edaravone removes lipid peroxides and hydroxyl radicals during ischaemia to provide neuroprotection from free radicals. With regard to potential utility in ALS, nitrotyrosine (a marker of oxidative stress) was detected in the spinal cord of SOD1-mutant mouse models and in the spinal cords of patients with familial SOD1-mutant ALS, suggesting that this compound might be involved in the neurodegenerative cascade.

Clinical trials of edaravone were undertaken in patients with ALS in Japan under the premise that elimination of free radicals could promote protection of motor neurons. A series of phase III studies of edaravone culminated in a successful FDA application in 2017 that centred around the potential benefit of edaravone in ALS when therapy commences in the early disease stages, within 2 years of onset, in

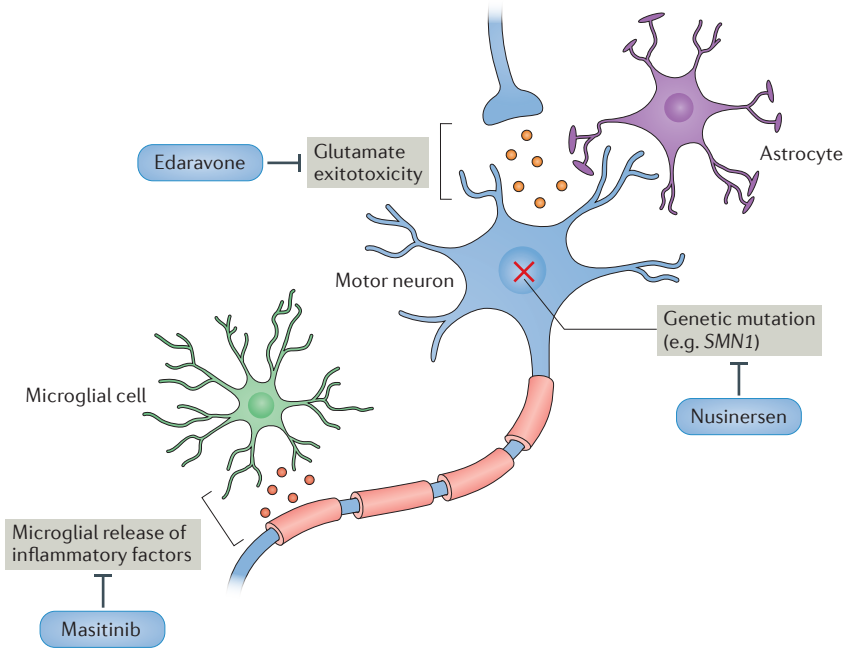

Figure 1 | **Therapeutic advances across the motor neuron disease landscape.** New therapies target disease mechanisms involved in a neurodegenerative cascade, from genetic origins through to excitotoxicity and neuroinflammation.

Key advances

- Antisense oligonucleotide therapy to increase the levels of functional survival motor neuron protein (SMN) in patients with spinal muscular atrophy resulted in improved motor milestones[2]
- Edaravone was approved by the FDA as a new treatment for amyotrophic lateral sclerosis (ALS) and demonstrated benefit in early disease stages[3]
- Large-scale genomic approaches confirmed a link between ALS and schizophrenia, suggesting the presence of shared neurobiological mechanisms[7]
- New research suggests that patient subgroups might have benefited in clinical trials that were previously considered to be negative, heralding the advent of precision medicine in ALS[10]

patients with a forced vital capacity of >80%[3]. Subsequent analysis of trial data estimated that this population of individuals with ALS typically accounts for 7% of patients who attend specialized clinical services. Although edaravone shows potential for benefit, further studies are likely to be required to satisfy international pharmaceutical regulatory authorities, particularly in relation to determining quality-of-life benefit.

Early trial data for masitinib — a tyrosine kinase inhibitor with action against microglia — were also announced for patients with ALS this year. Results of the phase II/III trial were outlined at the European Network for the Cure of ALS (ENCALS) meetings, confirming that the study had met its pre-specified end point[4]. Further analysis will be required, particularly in light of the negative findings of inflammatory therapies in ALS (including the results of the ozanezumab trial that were released during 2017 (REF. 5)) that highlight the importance of careful interpretation of study data and the need for development of improved disease biomarkers.

To facilitate therapeutic progress, ALS researchers will need to expand the repertoire of sensitive, valid and responsive outcome measures for clinical trial development to better understand the effects of treatments. The major outcome measures in current clinical research lack linearity, sensitivity and do not provide appropriate mechanistic markers of disease severity and progression. Clearly, the selection of responsive outcome measures for clinical trial development will be crucial to the future success of trials of therapies for ALS. Although biomarker approaches can seem highly complex in some instances (for example, detection of neurofilament levels in cerebrospinal fluid), others might become apparent as part of routine patient safety screening. Indeed, this year, plasma creatinine has been recognized as a potentially useful surrogate marker for neurodegeneration in ALS. Through the combination of some 13,000 measurements of creatinine from the LITRA study, EMPOWER study and the PROACT database, creatinine was found to usefully predict ALS functional status, muscle strength and mortality risk[6].

To date, the focus of ALS therapies has been on halting disease progression in the motor system. However, neurodegenerative disorders increasingly are viewed as multidimensional conditions that lead to deficits in motor function, cognition, mood and behaviour. Furthermore, extensive clinical, pathological and genetic overlaps exist between neurodegenerative disorders. ALS progression typically is charted in terms of degeneration of motor neurons in the brain and spinal cord, which leads to weakness. However, 80% of patients with ALS demonstrate behavioural and cognitive symptoms, and 15% of patients develop dementia. Common genetic causes between motor and cognitive disorders have been identified — such as repeat expansions in *C9orf72* — that result in ALS and/or frontotemporal dementia, underscoring the pathological overlap between these diseases.

Understanding the links between genetics, pathology and clinical phenotype across the spectrum of neurodegenerative disease will be crucially important to enable a combined approach to management that ensures that multisystem features such as cognitive deficits, behavioural dysfunction and motor degeneration are appropriately addressed. Furthermore, shared disease mechanisms might exist across the diseases of the brain and mind. A large international genetics consortium uncovered links between the genetically complex and heterogeneous syndromes of ALS and schizophrenia, which suggests the presence of shared neurobiological mechanisms and raises further tangible hope of new avenues for therapeutic progress[7].

Integrated multidisciplinary treatment has set the benchmark and standard of care in ALS. Multidisciplinary care pathways that involve specialist neurologist management in combination with allied disciplines, such as nursing, physiotherapy, occupational therapy and neuropsychology, have been demonstrated to improve quality of life and survival in patients with ALS[8]. Treatment and management of these multisystem deficits is central to the improvement of patient care and quality of life[9]. Integrated multidisciplinary care now has the potential to enable delivery of targeted interventions to reduce functional disability in neurodegenerative disorders. However, identification of defined clinical phenotypes is required to tailor treatment and management strategies to individual patients. As such, we are entering into a realm of precision medicine; indeed, this year, retrospective analysis of the overall negative outcomes from randomized trials that investigated the efficacy of lithium carbonate in patients with ALS (including a post hoc analysis of genetic data) found that treatment with this agent improved survival from 40.1% to 69.7% in patients with ALS who carried an *UNC13A* risk allele[10]. Such a finding might also be relevant to the many negative studies of ALS therapies that have been reported since the advent of riluzole. Regardless, we are now witnessing substantial evolution across the realms of clinical neurology and technology, which holds realistic promise for the emergence of new therapy and more critical analysis of emerging concepts regarding disease pathophysiology.

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EPILEPSY IN 2017

Precision medicine drives epilepsy classification and therapy

Sameer M. Zuberi and Andreas Brunklaus

2017 saw the publication of new classifications for epilepsy and seizure types, which emphasize the importance of understanding the underlying disease mechanisms. This aetiology-based approach is already beginning to inform developments in therapies and trial design in the epilepsies.

In 2017, personalized, stratified and precision medicine themes came to the fore in epilepsy research and practice, aided by important advances in epilepsy classification. Following an iterative process lasting over 10 years, an international collaborative effort led by the International League Against Epilepsy (ILAE) resulted in a new classification of epilepsies and seizure types, in which the inclusion of aetiology alongside clinical features was the principal development. The terminology for the new classifications was developed through engagement with the epilepsy community via online responses to a series of published proposals and at ILAE meetings, before the final publication of two ILAE position papers[1,2] and an accompanying instruction manual[3].

Key advances

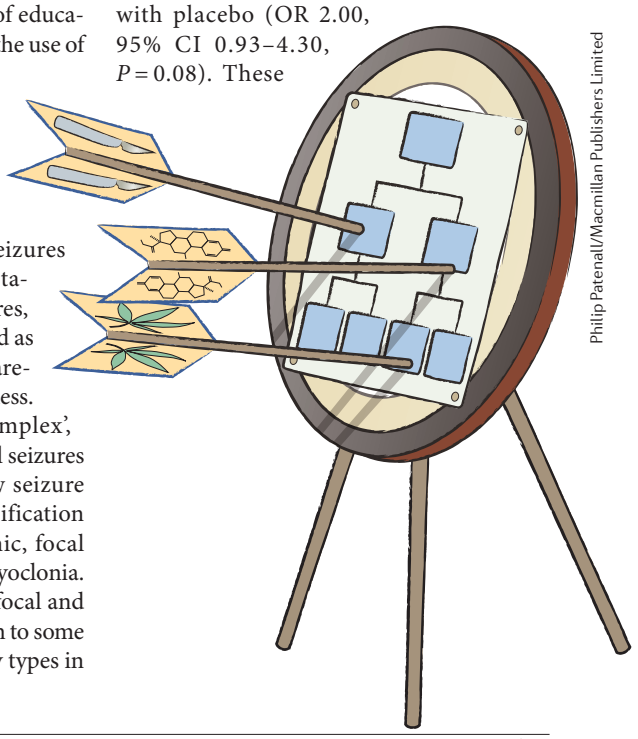
- The International League Against Epilepsy (ILAE) published a revised flexible framework for classification of the epilepsies, combining a clinical and aetiological approach and encouraging an individualized approach to therapy[1]
- The ILAE also published an updated classification of seizure types, introducing types not present in previous classifications and making the terminology more accessible to people with epilepsy and their carers[2]
- An international trial in children with Dravet syndrome, a rare genetic epilepsy syndrome, provided the first robust evidence of the efficacy of a botanically derived purified cannabinoid pharmaceutical in epilepsy[6]
- A randomized trial of surgical versus medical treatment in children with drug-resistant epilepsy revealed increased levels of seizure freedom in the surgical group and further deterioration in children who received medical treatment alone[8]
- The combination of vigabatrin and hormonal therapy in infantile spasms is more effective than hormonal therapy alone, particularly in patients with no identifiable aetiology[10]

The last official ILAE classification of seizures and epilepsies was published in 1989 (REF. 4). Since then, advances in genomic and neuroimaging technology have dramatically increased our understanding of the causes of epilepsies, and new aetiological subgroups — most notably, epilepsies related to immune dysfunction — have been defined. The new ILAE classification is a multilevel flexible framework that is designed to be applicable to all health-care settings[1,2]. The clinical features of epilepsy are defined at three levels: the seizure type, the epilepsy type and the epilepsy syndrome. At each level, the clinician is asked to consider the aetiology of the epilepsy, using six broad categories — structural, genetic, infectious, metabolic and immune — each of which can influence treatment choice. Epilepsies with unknown aetiologies are classified as such, and terms that have long been mysterious to people with epilepsy and other health professionals, such as 'cryptogenic', are now discouraged. The medical literature is no longer confined to the libraries of educational and medical institutions, and the use of accessible, straightforward language should aid communication between clinicians and people with epilepsy.

In the ILAE 2017 classification, seizures are classified as being of focal, generalized or unknown onset and are further subdivided into seizures with motor and nonmotor manifestations[2]. In the context of focal seizures, the term 'impaired awareness' is used as a surrogate for impairments of awareness, consciousness and responsiveness. Terms such as 'dyscognitive', 'complex', 'simple' and 'partial' to describe focal seizures are no longer recommended. New seizure types that feature in the 2017 classification include focal myoclonic, focal tonic, focal spasms, and absences with eyelid myoclonia. The orthodox dichotomy between focal and generalized epilepsies is broken down to some extent by the recognition of epilepsy types in which both focal andgeneralized seizures occur in the same individual.

2017 also saw the publication of a number of important trials of pharmacological and surgical therapies for rare and difficult-to-treat epilepsies. *Cannabis sativa* was the first plant cultivated by humans for purposes other than food, and for thousands of years, extracts of the plant have been used for a variety of therapeutic purposes, including the treatment of epilepsy. In recent years, case reports have appeared in the mainstream media — amplified by the power of new social media — purporting to demonstrate dramatic benefits of cannabis-based formulations for drug-resistant epilepsy, most notably a severe genetic developmental and epileptic encephalopathy of childhood called Dravet syndrome. These reports led to a demand from families for a treatment for which no strong evidence base existed, and unprecedented numbers of children in the USA were placed on an open-label, compassionate-use programme for cannabidiol, a botanically derived pharmaceutical[5].

In 2017, the results of a double-blind, placebo-controlled trial of cannabidiol in children with Dravet syndrome were published[6]. Dravet syndrome is rare, with an estimated incidence of 1 in 16,000 live births, but by undertaking the trial in multiple centres in Europe and North America, the investigators were able to recruit 120 children and record sufficient seizures to detect a difference between placebo and cannabidiol treatment[7]. The percentage of patients who had a greater than 50% reduction in convulsive seizure frequency was 43% with cannabidiol and 27% with placebo (OR 2.00, 95% CI 0.93–4.30, $P = 0.08$). These



Philip Paternali/Macmillan Publishers Limited

results have gone some way towards tempering the hype and unrealistic expectations around cannabis-based therapies for the epilepsies, and this trial will act as a model for future syndrome-specific trials in rare diseases.

> “...seizure freedom seemed to outweigh the neurological deficits experienced after surgery”

Another trial with innovative methodology, which compared an early surgical treatment group with a group in which surgery was offered following a trial of further medical therapies, has strengthened the evidence for early epilepsy surgery as a curative treatment for drug-resistant epilepsy in children and adolescents. In a cohort of 116 children and adolescents with a clear epileptic focus and no history of status epilepticus, Dwivedi *et al.* compared epilepsy surgery ($n = 57$) with continued medical therapy alone ($n = 59$)[8]. At 12 months, 44 patients (77%) in the surgery group had complete seizure freedom compared with only 4 patients (7%) in the medical therapy group. A substantial proportion of children in the surgical group (33%) were left with a marked neurological deficit (monoparesis or hemiparesis), which improved over time in some cases. Despite these impairments, children in the surgical group had significantly improved social quotient, behavioural and quality-of-life scores after 1 year, whereas these measures deteriorated in the medical therapy group. Overall, seizure freedom seemed to outweigh the neurological deficits experienced after surgery. In addition to showing that surgery improves outcomes in eligible children, this study emphasizes that further disease-related deterioration occurs in patients who do not undergo surgery.

Good surgical results can also be achieved in patients with nonlesional epilepsy, as shown by Kim *et al.* in a recent study[9]. In a cohort of 109 consecutive patients without identifiable lesions on 1.0T or 1.5T MRI who underwent focal surgical resection, nearly 60% achieved seizure freedom 10 years after surgery. Several factors seemed to predict a favourable surgical outcome, including localizing patterns on functional neuroimaging (^{18}F-FDG–PET and ictal single-photon emission CT), the presence of aura, and concordant results on presurgical assessments.

Epileptic spasms in infancy merit urgent assessment and rapid treatment, as delays can be associated with poor cognitive outcomes. The two best-established therapies are hormonal treatments (steroids or adrenocorticotropic hormone) and vigabatrin; however, little international consensus exists on which treatment to use first. In 2017, O'Callaghan and colleagues reported the results of International Collaborative Infantile Spasms Study (ICISS), the largest open-label randomized trial for the treatment of infantile spasms to date. For this multinational trial, 102 hospitals in five countries screened 766 infants over a 7-year period, 377 of whom were recruited to the study[10]. The infants were randomly assigned to hormonal monotherapy or combination treatment consisting of hormone therapy and vigabatrin. The strict primary outcome was no spasms between days 14 and 42. This outcome was achieved in 72% of infants in the combined treatment arm compared with 57% of those on hormonal therapy alone. The treatment response was more marked in infants with no identifiable aetiology. The earlier the treatment was started, the better the response, emphasizing the need to treat infantile spasms promptly and aggressively with combination therapy.

Well-controlled, randomized, large-scale trials of novel and established therapies are providing strong evidence to guide therapeutic choices in epilepsy. In particular, these trials highlight the importance of early intervention and aetiology-driven classification so as to ensure precise targeting of the most appropriate treatments.

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STROKE IN 2017

Intensive and extensive — advances in stroke management

Meng Lee and Bruce Ovbiagele

The past year saw advances in endovascular treatment for acute stroke, speech therapy for aphasia after stroke, and cardiac disease management to prevent stroke. These treatments were characterized by more intensive or more extensive effects than standard care, necessitating thoughtful translation of the clinical trial findings into routine clinical practice.

The year 2017 has seen several advances in the management of stroke, encompassing a variety of therapeutic approaches. The evidence is diverse, ranging from support for wider use of existing approaches to the first support for use of new approaches. The approaches themselves are also varied, covering the spectrum from prevention to rehabilitation. Each area, however, promises to improve outcomes for patients with stroke.

Important evidence published in 2017 has provided further insight into the benefits of endovascular thrombectomy for patients with acute ischaemic stroke. The efficacy of

endovascular thrombectomy within 8 h of acute ischaemic stroke onset to improve 3-month functional outcomes among patients with large artery occlusion in the anterior cerebral arterial circulation was convincingly proven in 2015. However, whether the benefits of endovascular thrombectomy in these patients are sustained in the long term, and whether the therapeutic window for endovascular thrombectomy could be extended beyond 8 h, remained unclear.

In 2017, follow-up data from the MR CLEAN trial were published, reporting 2-year outcomes after patients were randomly assigned to either endovascular treatment (intervention group) or conventional treatment (control group) for acute ischaemic stroke[1]. Of the 500 patients involved in the original trial, 2-year data were available for 391 patients. The distribution of outcomes on the modified Rankin scale favoured endovascular treatment over conventional treatment (adjusted common OR 1.68, 95% CI 1.15–2.45). Patients in the intervention group were more likely than patients in the control group to have a good outcome (modified Rankin scale score 0–2; adjusted OR 2.21, 95% CI 1.30–3.73) and to have a favourable outcome (modified Rankin scale score 0–3; adjusted OR 2.13, 95% CI 1.30–3.43). The cumulative 2-year rate of death did not differ between the intervention and control groups. Quality of life was better among patients who received endovascular treatment than in patients who received conventional treatment, as assessed with the European Quality of Life 5 Dimensions questionnaire (mean score difference 0.10, 95% CI 0.03–0.16). The beneficial effect of endovascular treatment on functional outcome at 2 years in patients with acute ischaemic stroke was similar to that reported at 90 days in the original trial. These follow-up data reinforce the findings of the initial trials and show that patients who receive endovascular thrombectomy continue to benefit in the long term.

> The results of the DAWN trial … support more widespread use of endovascular thrombectomy…

The DAWN trial examined whether endovascular thrombectomy provides benefits beyond standard care in patients with occlusion of the intracranial internal carotid artery or proximal middle cerebral artery, who were last known to be well 6–24 h earlier, and in whom the severity of clinical deficit and infarct volume were mismatched[2]. The trial was stopped because a prespecified interim analysis revealed overwhelming benefits of thrombectomy plus standard care when compared with standard care alone. The rate of functional independence (defined as a modified Rankin scale score of 0–2) at 90 days was 49% in the thrombectomy group and 13% in the control group (adjusted difference 33 percentage points, 95% CI 24–44 percentage points). The rate of symptomatic intracranial haemorrhage and mortality did not differ significantly between the two groups[2]. The results of the DAWN trial, therefore, support more widespread use of endovascular thrombectomy for patients with acute ischaemic stroke, even if the time since onset is longer than the window that is widely accepted to provide benefit.

In preventive stroke treatment, the role of patent foramen ovale (PFO) closure in reducing recurrent ischaemic stroke has been controversial, but three randomized trials published in 2017 have provided new evidence that all but closes this debate. The CLOSE[3], Gore REDUCE[4], and RESPECT-Extended[5] trials compared the efficacy of transcatheter PFO closure with that of medical therapy for the prevention of recurrent ischaemic stroke among patients with a PFO and cryptogenic stroke or transient ischaemic attack. All three trials used double-disc devices for PFO closure, and all showed that PFO closure was associated with lower risks of recurrent stroke than was medical therapy (HR 0.03, 95% CI 0.00–0.26 for the CLOSE trial[3]; HR 0.23, 95% CI 0.09–0.62 for the Gore REDUCE trial[4]; and HR 0.55, 95% CI 0.31–1.00 for the RESPECT-Extended trial[5]). The benefit of PFO closure was greatest among patients with an atrial septal aneurysm and a large right-to-left shunt.

The concurrence between the findings of the three trials provides strong evidence to settle the debate and demonstrate the benefit of PFO closure, although the incidence of atrial fibrillation (AF) was greater among patients who underwent PFO closure in these trials. Further study of the incidence of AF after PFO closure (which was mostly transient) is indicated, but should not preclude the routine use of this treatment, especially for patients with high-risk PFO characteristics, such as atrial septal aneurysm and a large right-to-left shunt.

> …the role of patent foramen ovale … closure in reducing recurrent ischaemic stroke has been controversial…

For the prevention of stroke in patients with nonvalvular AF, non-vitamin K antagonist oral anticoagulants (NOACs) are proven to be as effective as and safer than warfarin. The major trials of NOACs have excluded patients with AF who have mitral stenosis or mechanical heart valves, but have included patients with other native valve pathologies, making it difficult for practitioners to decide on which therapy is best for patients with valvular disease. Published in 2017, a substudy of the ENGAGE AF-TIMI 48 trial showed that use of the NOAC edoxaban rather than warfarin was associated with a trend towards a lower risk of stroke or systemic embolism (HR 0.69, 95% CI 0.44–1.07) and a lower risk of intracranial haemorrhage (HR 0.39, 95% CI 0.15–0.98) among patients with valvular AF[6]. Furthermore, a meta-analysis that combined all data from four major trials of NOACs versus warfarin showed that NOACs reduced the risk of stroke or systemic embolism (HR 0.70, 95% CI 0.60–0.82) and intracranial haemorrhage (HR 0.47, 95% CI 0.24–0.92) in patients

Key advances

- Follow-up data from the MR CLEAN trial showed that the benefits of endovascular thrombectomy for acute ischaemic stroke over conventional treatment remain after 2 years[1]
- The DAWN trial showed that endovascular thrombectomy can benefit patients whose onset of stroke symptoms was 6–24 h earlier and whose clinical deficits did not match the infarct volume[2]
- Patent foramen ovale closure after stroke was shown to reduce the risk of recurrent stroke during extended follow-up[5], all but closing the debate about the benefits of this treatment
- A meta-analysis of trials showed that non-vitamin K antagonist oral anticoagulants lower the risk of stroke, systemic embolism and intracranial haemorrhage in patients with valvular atrial fibrillation when compared with warfarin[7]
- A trial of intensive speech and language therapy revealed improvements in patients aged ≤70 years with chronic aphasia after stroke[8]

with valvular AF[7]. Of note, however, the reduction in risk of intracranial haemorrhage was driven by apixaban, edoxaban and dabigatran (HR 0.33, 95% CI 0.25–0.45); rivaroxaban did not have the same effect (HR 1.27, 95% CI 0.58–2.79)[7].

After a stroke, spontaneous recovery of linguistic function is generally accepted to occur mostly within the first few weeks, and to be complete within the first year. Evidence that indicates efficacy of a treatment strategy for chronic aphasia (≥6 months after stroke) is non-existent, but one trial published in 2017 produced promising results. This randomized, open-label, blinded-end-point, controlled trial examined whether 3 weeks of intensive speech and language therapy under routine clinical conditions improved verbal communication in daily-life situations in patients aged ≤70 years with chronic aphasia after stroke[8]. Verbal communication significantly improved from baseline after the treatment (mean difference 2.61 points, 95% CI 1.49–3.72)[8].

In conclusion, several studies published in 2017 showed that various intensive and extensive therapies improved stroke outcomes. The benefits of endovascular thrombectomy have now been proven in both the short term and the long term. This approach was also shown to be effective more than 8 h after acute ischaemic stroke onset, and in patients with a clinical–imaging mismatch. Therefore, more widespread systematic use of this therapy in appropriate patients is warranted. The controversy surrounding the ability of PFO closure to reduce recurrent ischaemic stroke has been laid to rest, especially in certain patient subgroups, and the evidence supports routine implementation of this treatment in clinical practice. In addition, on the basis of emerging evidence, NOACs might be a reasonable alternative to warfarin for reducing the risk of stroke in patients with AF who have native valvular heart disease. We now also have initial evidence to support intensive and attentive treatment of chronic aphasia after stroke, although more trials are needed to clarify the minimum treatment intensity required for meaningful clinical effect.

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Competing interests statement
The authors declare no competing interests.

PARKINSON DISEASE IN 2017

Changing views after 200 years of Parkinson disease

Walter Maetzler and Daniela Berg

200 years after James Parkinson's An Essay on the Shaking Palsy, 2017 has seen important advances that are driving a shift towards a broader and more holistic understanding of Parkinson disease aetiology and progression. This shift might finally pave the way to entirely novel and more effective prevention and management strategies.

In 2017, the 200th anniversary of James Parkinson's description of the disease that was to be named after him, the main goal of Parkinson disease (PD) research remains finding strategies for a cure. An exhaustive review published this year[1] defined the most promising strategies: implementation of precision medicine into the development of treatment for PD; further characterization of PD phases, including the prodromal phase; improvement of neuroimaging and molecular genetics approaches to increase understanding of how PD begins and progresses; more extensive implementation of new wearable sensor technology; and (big) data management approaches. The past year has seen advances as a result of many of these strategies, and the findings are starting to be combined to strengthen the case for developing new approaches to the classification and management of PD.

Perhaps the clearest example of how out-of-the-box thinking is changing our view of PD was a provocative review[2] in which a new precision medicine approach to the development of disease-modifying strategies was proposed. The authors argue in favour of a "biomarker-driven phenotype" approach to defining cohorts for trials in PD instead of the current most frequently used approach of searching for "phenotype-driven biomarkers" (FIG. 1).

The usefulness of the biomarker-driven phenotype approach is supported by pathomechanistic studies published in 2017 that highlight the relevance of mechanisms that can be involved in both neurodegeneration and immunological responses. For example, one study[3] identified an intriguing mechanism by which mutated microglia could induce neurodegeneration. Mice with a cancer-causing mutation in the *Braf* gene developed the expected myeloid cell tumours only when the mutated cells originated in embryos. If the mutated cells originated in the yolk sac, the animals developed behavioural, biochemical and pathological features of late-onset neurodegeneration. As somatic mutations in this gene (and others) can arise spontaneously and lead to mosaicism in distinct lineages, the findings raise the intriguing idea that mutations in cells derived from the blood system can contribute to the pathogenesis of common forms of late-onset neurodegenerative diseases such as PD.

A large genome-wide association study published in 2017 further supports the idea that a specific association exists between autoimmune diseases and PD[4] and, thus, widens our understanding of common mechanisms resulting in phenotypically distinct diseases. The authors identified 17 genetic loci that are each involved in PD and various autoimmune diseases, probably reflecting

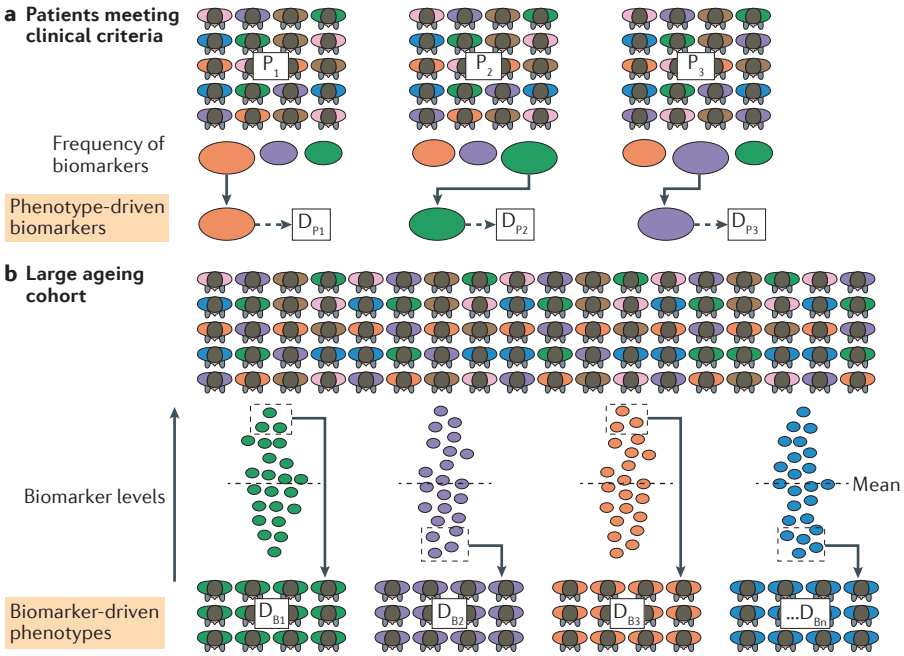

Figure 1 | **Precision medicine in Parkinson disease.** Proposal for a novel precision medicine approach that could be particularly useful for the development of disease-modifying strategies for Parkinson disease. **a** | Conventional approaches for the definition of cohorts use phenotype (P)-driven strategies. **b** | A biomarker (B)-driven strategy might be more effective. In this strategy, obvious disease (D)-associated biological signals are examined on an exploratory basis within large cohorts, including people with other diseases and healthy individuals. Biomarker-driven phenotypes (D_{B1}–D_{Bn}) can then be defined by, for example, biomarker levels that are more than two standard deviations above or below the mean. Adapted with permission from REF. 2, John Wiley & Sons.

common genetic pathways and implying that future therapeutic trials in PD could include anti-inflammatory agents, at least for some stratified patients.

Many research groups have pursued strategies to improve characterization of the prodromal phase of PD, including some that have published validation studies of the recently introduced Movement Disorders Society research criteria, which define the probability of prodromal PD. According to a study in which two large cohorts were combined[5], the specificity and negative predictive value of these criteria are high, whereas their sensitivity and positive predictive value are low. These results highlight the need for broader, more specific and objective diagnostic testing to select individuals at high risk of PD to participate in the urgently needed intervention trials in prodromal PD. Such testing might require the identification of additional and more powerful markers of prodromal PD and, potentially, the use of a stepwise screening approach.

In clinical PD research, one study presented an important step towards a more efficient, personalized approach to clinical trials and treatment strategies[6]. In this study, 421 patients with *de novo* PD from the Parkinson's Progression Markers Initiative were classified into three subtypes on the basis of a motor summary score and three nonmotor features (cognitive impairment, REM sleep behaviour disorder and dysautonomia) assessed at baseline. Patients with the 'diffuse malignant' subtype exhibited a greater decline in cognition and dopaminergic function than patients with the other two subtypes ('mild motor-predominant' and 'intermediate') after an average of 2.7 years. Moreover, patients with the diffuse malignant subtype already exhibited a more profound dopaminergic deficit, greater atrophy in PD brain networks and a more Alzheimer disease-like cerebrospinal fluid profile at baseline. In combining individual biomarkers with assessment of disease progression, this study presents a promising framework for the development of more specific definitions of PD subtypes.

PD includes motor and nonmotor aspects, and increasing evidence suggests that the degeneration of non-dopaminergic structures is largely responsible for nonmotor features. Nonmotor symptoms have become a greater focus for researchers and clinicians because they are often relevant to daily life and can severely affect health-related quality of life[7]. Moreover, degeneration of non-dopaminergic structures seems to influence obvious motor symptoms, with therapeutic implications. One example is the loss of gait automaticity associated with cholinergic dysfunction. A recent randomized, placebo-controlled, double-blind, parallel-arm trial[8] in patients with PD found that the cholinesterase inhibitor rivastigmine improved step time variability and reduced fall rates during normal walking and in a simple dual-task walking test. This finding is important because many patients with PD fall, and current treatment strategies for gait deficits in PD are unsatisfactory.

Efforts to develop more precise neuroimaging led to the publication in 2017 of an interesting approach to improve our understanding of the interplay between automaticity and dual-tasking deficits in PD[9]. The study shows that during dual tasking, patients with PD engage the ventroposterior putamen, a striatal territory that is not engaged in

Key advances

- A mechanism was identified in which microglia with a cancer-causing mutation could induce neurodegeneration[3], prompting out-of-the-box thinking about possible starting points and origins of neurodegeneration
- Validation of the Movement Disorders Society research criteria for prodromal Parkinson disease (PD) demonstrated high specificity but relatively low sensitivity and positive predictive value[5]; improvements have been proposed
- Subtyping of PD on the basis of biomarkers and imaging, including parameters reflecting disease progression, provided a promising novel framework for specific and treatment-relevant definitions of PD subtypes[6]
- Dual tasking in PD was found to be associated with reduced segregation of striatal areas[9], suggesting that treatment for dual-tasking deficits should aim to improve segregation in the basal ganglia
- An animal study demonstrated that the β2-adrenoreceptor regulates α-synuclein expression; database analysis showed that PD risk in humans was decreased by treatment with β2-adrenoreceptor agonists and increased by antagonists[10]

patients during single cognitive or motor tasks or in controls during the single tasks or dual tasking. The investigators interpreted this finding to mean that dual-tasking deficits in PD might be caused by "functional blurring between loops that normally process information in parallel" — effectively, a reduced spatial segregation of neighbouring striatal territories. As a consequence, future treatment should focus on increasing this striatal segregation instead of optimizing the respective tasks.

Continued intense α-synuclein research in 2017 has also brought new insights with implications for PD treatment. In particular, one study in mice with neurotoxin-induced parkinsonism and induced pluripotent stem cell-derived neuronal cultures showed that increasing the levels of the β2-adrenoreceptor decreases expression of the α-synuclein gene (*SNCA*)[10]. In the same study, analysis of a database including 4.6 million Norwegians who were observed over 11 years revealed that treatment with the β2-adrenoreceptor agonist salbutamol reduced the risk of developing PD by ~30%, whereas treatment with the β2-adrenoreceptor antagonist propranolol increased the risk twofold. This study provides strong arguments that compounds designed to reduce the endogenous transcription of *SNCA* can benefit humans. Moreover, the findings clearly demonstrate the value of registers and the potential of drug-repurposing strategies.

Collectively, research published in 2017, together with ongoing work, is broadening our understanding of the complexity and heterogeneity of PD pathogenesis, thereby highlighting the need for new ways of thinking about the disease and new approaches to its management. This change in thinking will have important implications for more personalized and powerful treatment and management strategies in the future.

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Competing interests statement
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MULTIPLE SCLEROSIS IN 2017

Progress in multiple sclerosis — from diagnosis to therapy

Maria Trojano and Maria Pia Amato

In 2017, extensive research into multiple sclerosis (MS) resulted in improved diagnostic criteria, development of biomarkers that enable monitoring of disease evolution and treatment response over time, and identification of novel genetic markers of disease susceptibility. In addition, 2017 saw the first successful clinical trials of remyelination strategies and treatments for progressive MS.

A 2017 revision of the McDonald criteria for multiple sclerosis (MS)[1] has been published by the International Panel on Diagnosis of MS. The new criteria aim to facilitate earlier diagnosis of MS by simplifying or clarifying components of the previous revisions while retaining their specificity. The Panel emphasized caution regarding possible misdiagnosis[2] and recommended careful attention to alternative diagnoses and comorbidities — especially within certain populations such as African-American, Asian and Latin American individuals as well as in children and elderly people.

The Panel made a number of changes to previous criteria, supported by the accumulation of substantial evidence-based data. The new criteria state that the presence of cerebrospinal fluid (CSF)-restricted oligoclonal bands can be a substitute for fulfilling the requirement of dissemination in time (DIT) — and, consequently, can be used to make a diagnosis of MS — in patients who have a typical, clinically isolated syndrome and clinical or MRI demonstration of dissemination in space (DIS). In addition, symptomatic lesions (and not merely asymptomatic lesions) can now be used to demonstrate DIS and/or DIT. Furthermore, cortical lesions, in addition to juxtacortical ones, can be used to demonstrate DIS. The diagnostic criteria for primary progressive MS (PPMS) remain unchanged, except for the inclusion of cortical lesions and symptomatic MRI lesions. These revised criteria strengthen the diagnostic role of CSF analysis and validate the use of MRI features that were not included in the previous criteria[3]. In the future, the Panel should re-examine the diagnostic contribution of optic nerve involvement, advanced imaging, and neurophysiological and body fluid markers.

In addition to the available MRI-based measures of MS, the development and validation of body fluid markers that reflect tissue damage remains an important area of investigation to support early and accurate diagnosis of MS, monitoring of treatment response and prediction of long-term prognosis. To date, CSF levels of neurofilament light chain (NFL) are among the most promising markers of inflammation and neurodegeneration in MS[4]. However, CSF sample collection is invasive and constitutes a burden to the patient, which prevents longitudinal assessments. The development of highly sensitive immunoassay techniques could enable the determination of extremely low quantities of NFL in serum. Disanto and colleagues[5] reported the value of a novel, ultrasensitive single-molecule array (Simoa) assay for the measurement of serum levels of NFL in MS. The Simoa assay yielded a 126-fold and 25-fold higher sensitivity for NFL than did enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence-based assays, respectively. Serum levels of

NFL were measured in healthy control individuals ($N=254$) and in independent, cross-sectional ($N=142$) and longitudinal ($N=246$) MS cohorts. The study found that serum and CSF levels of NFL were strongly correlated ($P<0.001$), indicating that blood sampling can replace CSF taps for detection of this marker. Moreover, serum levels of NFL were higher in patients with MS than in healthy controls ($P<0.001$) and were higher in patients with MS who had gadolinium-enhancing, T1-weighted MRI brain and spinal cord lesions ($P=0.002$) than in those who did not. Furthermore, serum levels of NFL were lower in patients with MS who were under disease-modifying treatment ($P<0.003$) than in untreated patients and correlated with previous, concurrent and future relapses as well as disability worsening. These results could represent a turning point in the search for new MS biomarkers and encourage further validation of serum NFL in randomized controlled trials.

> The ORATORIO study … was the first randomized controlled trial to meet its primary disability end point in PPMS

This year also saw advances in our understanding of the MS pathomechanisms. A genetic study by Steri and colleagues[6] provided information on disease mechanisms and drug-targetable pathways in MS with considerable translational potential. Using case–control samples from Sardinia, Italy, a genome-wide association study was performed in patients with MS. The researchers used phenotyping of quantitative immune variables, sequence-based fine mapping, cross-population and cross-phenotype analyses, and gene-expression studies to identify the MS-causative variant in this population and assess its mechanism of action. The results showed that a variant in *TNFSF13B*, encoding the cytokine B cell-activating factor (BAFF; also known as tumor necrosis factor ligand superfamily member 13B), was associated with an increased risk of both MS and systemic lupus erythematosus (SLE). The allele was associated with upregulated humoral immunity through increased levels of soluble BAFF, B lymphocytes and immunoglobulins. These findings support BAFF as a therapeutic target in MS and SLE, and are consistent with the efficacy of B cell-depleting therapies recently approved for MS.

This year has also seen considerable therapeutic advances. Following the revolution in the treatment of relapsing–remitting MS (RRMS) that has occurred within the past 5 years, the focus of MS research has now turned to the more challenging condition of PPMS.

The ORATORIO study[7] of ocrelizumab, published in 2017, was the first randomized controlled trial to meet its primary disability end point in PPMS. Ocrelizumab is a humanized monoclonal antibody that selectively depletes CD20-expressing B cells and preserves the capacity for B cell reconstitution and pre-existing humoral immunity. In the 732 patients with PPMS studied, 12-week confirmed disability progression was 32.9% with ocrelizumab versus 39.3% with placebo ($P=0.03$). Secondary end points, including 24-week confirmed disability progression, ambulation speed, change in brain volume and the total volume of brain lesions on T2-weighted MRI, also favoured ocrelizumab versus placebo.

This trial refutes the dogma that PPMS cannot be treated with anti-inflammatory agents and encourages further immunological research to decipher the pathogenic role of B cells in MS. Furthermore, the trial underscored the importance of careful selection of populations with PPMS, as it included only young patients (<55 years of age) with a shorter disease duration (<15 years) who have an increased likelihood of disease activity. Indeed, the design of ORATORIO was informed partly by the results of a previous trial of the chimeric, monoclonal, anti-CD20 antibody rituximab in patients with PPMS[8]. Although the previous study did not reach its primary disability end point, post hoc analyses showed delayed disability progression in younger patients who had evidence of active inflammation. Although the clinical benefit of ocrelizumab is modest, the ORATORIO trial stands as a major breakthrough in the treatment of PPMS.

2017 also saw the first demonstration of efficacy by a remyelinating treatment. The ReBUILD trial[9] was a phase II, single-centre, 150-day, crossover, randomized controlled trial that documented the efficacy of clemastine fumarate for the treatment of chronic demyelinating optic neuropathy in 50 patients with RRMS on stable immunomodulatory therapy. Clemastine fumarate is a first-generation antihistamine that has been shown to induce oligodendrocyte precursor differentiation and remyelination in vitro and in animal models, without modulating the immune system[9]. The primary efficacy end point was the shortening of P100 latency on full-field, pattern-reversal visual evoked potentials. The crossover model showed a reduction in latency of 1.7 ms per eye ($P=0.0048$), whereas a post hoc, delayed treatment model showed a reduction in latency of 3.2 ms per eye ($P=0.0001$) for the period on treatment. Moreover, during treatment, patients showed a trend towards improvement in low-contrast letter acuity (LCLA), which became significant when analysed with the delayed-treatment model.

As a result of the robust preclinical data on this therapy, the trial results support human efficacy of clemastine fumarate. Moreover, preliminary findings suggest that this electrophysiological effect might result in improved, clinically meaningful outcomes, as LCLA is a well-validated measure of visual function in MS[10]. Overall, this study supports further investigation of clemastine fumarate, provides a framework for future trials of remyelinating therapies in MS and highlights that the visual system is a suitable model for studying repair and remyelination in the disease, owing to its anatomical segregation and the precision of the clinical tests available for visual assessment. The study also suggests that the combination of remyelinating and immunomodulatory agents in MS treatment is clinically beneficial.

Key advances

- New 2017 diagnostic criteria for multiple sclerosis (MS) aim to facilitate early diagnosis, strengthen the validity of cerebrospinal fluid oligoclonal bands and cortical and symptomatic MRI lesions as diagnostic markers of MS, and recommend caution regarding possible differential diagnoses[1].
- New highly sensitive immunoassay techniques enable the measurement of serum levels of neurofilament light chain, which seems to be a promising biomarker of inflammation and neurodegeneration in MS[5].
- A genetic variant in *TNFSF13B* is associated with an increased risk of MS related to upregulated humoral immunity, indicating new, drug-targetable pathways[6].
- The ORATORIO study of ocrelizumab was the first phase III trial to meet its primary disability end point in primary progressive MS, representing a major breakthrough in the treatment of progressive MS[7].
- The ReBUILD trial of clemastine fumarate demonstrated efficacy of a remyelinating agent in patients with MS for the first time, providing a framework for future trials of remyelinating therapies in MS[9].

To conclude, remarkable progress has been made in different areas of MS research in 2017. The achievements discussed here have addressed some of the key issues in MS and could highlight the future directions of the field.

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Competing interests statement
The authors declare no competing interests.

INFLAMMATION IN 2017

Connectivity to other fields brings new ideas

Pierre Miossec

Multiple scientific fields pertaining to inflammation, including the fields of cardiovascular, infection and cancer research, are increasingly contributing to our understanding of rheumatoid arthritis (RA). In 2017, such research has helped develop our understanding of RA comorbidity, the link between RA pathogenesis and infection, and the effects of new therapies.

For decades, research in the field of arthritis, specifically in rheumatoid arthritis (RA), has focused on the joint-related aspects of disease pathogenesis. Such research began with efforts to understand how an immune inflammatory reaction could target the peripheral joints. Collagen type II was proposed as a causative antigen because of its presence in cartilage (the target site of destruction), and because arthritis is induced in mice following collagen type II administration. Although the presence of joint-specific target antigens provides a simple (but maybe too simple) explanation for RA pathogenesis, this research has not translated to new treatment approaches. Step by step, however, researchers are uncovering alternative explanations by looking at similar mechanisms in different types of inflammation affecting diverse organs in different diseases. In 2017, research in multiple fields outside of arthritis has contributed to our understanding of RA, including the cardiovascular[1], infection[2] and cancer fields[3,4], providing insight not only into disease pathogenesis but also into less frequently studied aspects of disease, such as RA comorbidities and the effects of new therapies (FIG. 1).

In the cardiovascular field, new results published in 2017 show that targeting pro-inflammatory cytokines in patients at high risk of cardiovascular disease protects against new cardiovascular events[1]. Patients with arthritis have a reduced life expectancy, owing in part to an increased frequency of cardiovascular events (namely myocardial infarction and, to a lesser extent, stroke). The major goal of RA treatment up until now has been to reduce, and possibly prevent, joint destruction. Although investigating the protective effect of RA treatment on cardiovascular events is complex, the current hypothesis is that treating inflammation as early as possible reduces the risk of cardiovascular events and at the same time better protects against joint destruction. For the general population, cardiovascular risk was originally thought to be a consequence of an altered lipid profile. This notion has led to massive efforts to target lipids, focusing on diet, salt intake and the use of statins to reduce the negative effect of lipids. However, patients with RA have a lipid profile normally associated with protective cardiovascular effects[5]. Paradoxically, controlling inflammation with one of a number of anti-inflammatory treatments leads to a less protective lipid profile; for example, as highlighted in 2017 with IL-6 inhibition[5]. Previous research into the mode of action of statins has provided some insight into this 'lipid paradox'. Statins are thought to protect against cardiovascular disease by lowering cholesterol levels; but, in addition to having lipid-lowering properties, statins also have large anti-inflammatory effects through the cholesterol pathway[6].

In the general population, the presence of any inflammation is a major cardiovascular risk factor. For example, a modest elevation in C-reactive protein level is strongly associated with an increased risk of cardiovascular events[1]. Based on this observation, patients with a previous myocardial infarction at high risk of another cardiovascular event were treated with an antibody against IL-1β (canakinumab) in a large double-blind trial (the CANTOS trial)[1]. The control of IL-1-driven inflammation reduced the incidence of cardiovascular events in these patients, independent of cholesterol level. Hence, the control of inflammation seems to be the key factor in reducing cardiovascular risk. Canakinumab mediated its effects through targeting circulating IL-1β, which confirms previous findings from many years ago that vessel-lining endothelial cells produce IL-1β[7]. In the CANTOS trial, the occurrence of lung cancer was also reduced in treated patients, indicating that cancer could also be linked to inflammation[8]. In line with the role of cytokines in host defence, however, patients treated with canakinumab also had an increased frequency of systemic infections.

In the field of infection, new findings from 2017 provide further insight into RA pathogenesis, suggesting that mechanical stimuli cooperate with buccal infections to induce anti-cyclic citrullinated peptide (anti-CCP) autoantibody production[2]. The causal role of infections in RA pathogenesis has been suspected but never fully proved. Various explanations have been proposed for this relationship. Currently, a popular concept to explain the occurrence of anti-CCP antibodies concerns the cross-reactivity of bacterial enzyme-modified protein antigens in the mouth, lung or gut with joint antigens. Interestingly, mastication itself induces a local inflammatory reaction in the mouth, leading to periodontitis[2]. Such local inflammation induces the production of IL-6, which in turn is a key inducer of T helper 17 (T_H17) cell differentiation. The net effect is a local activation of T_H17 cells and other IL-17-producing cells, which contribute

Key advances

- The occurrence of infection in combination with mechanical stress leads to increased local T helper 17 cell activation, which might ultimately result in the induction of anti-cyclic citrullinated peptide (anti-CCP) antibodies[1].
- Inhibition of IL-1 protects against cardiovascular events in a population at high risk of cardiovascular disease but without arthritis[2].
- Induction of apoptosis in mesenchymal cells protects against arthritis[4] but activation of T cells during cancer treatment might lead to arthritis[3].

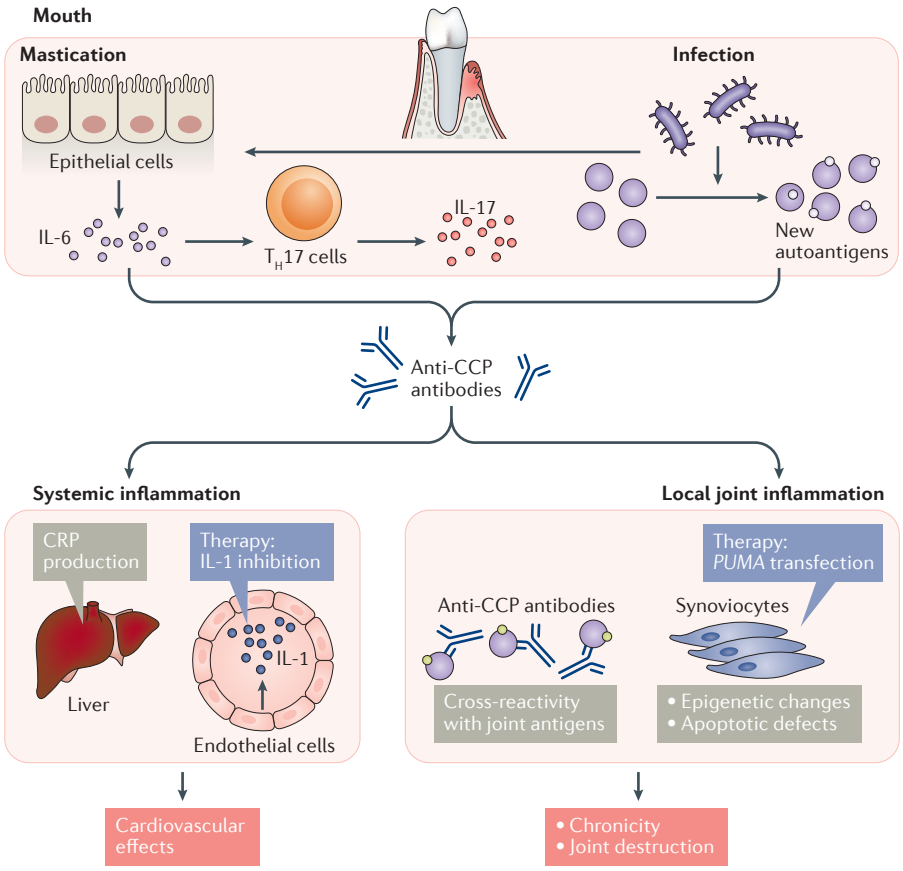

Figure 1 | **Contributions from the cardiovascular, infection and cancer fields to the rheumatoid arthritis field.** Research in the fields of cardiovascular, infection, and cancer research in 2017 has brought new insight to the understanding and treatment of rheumatoid arthritis (RA). A combination of mechanical stimuli during mastication and bacterial infections in the mouth leads to inflammation and IL-6 secretion. This effect favors local T helper 17 (T_H17) cell differentiation and IL-17 secretion, as well as the induction and production of anti-cyclic citrullinated peptide (anti-CCP) antibodies, through infection-induced changes in protein structure. Cross-reactivity of these antibodies with antigens in the joints leads to local joint inflammation. The very same cytokines involved in arthritis contribute to the increased risk of cardiovascular events. Inhibition of IL-1 in patients at high risk of cardiovascular disease but without arthritis protects from recurrence of cardiovascular events. Chronic joint inflammation leads to apoptotic defects in synoviocytes. Local administration of the *PUMA* proapoptotic gene protects against arthritis and joint destruction. CRP, C-reactive protein.

to local bone resorption, with IL-17 amplifying TNF, IL-1 and IL-6-driven inflammation and bone resorption. Researchers have previously implicated the T_H17 pathway in the early induction of anti-CCP autoantibodies[9]. Hence, this mechanism might also amplify the induction of anti-CCP antibodies. However, the sequence and link between local infection, inflammation, autoantibodies and clinical RA remains to be established.

Whether T cells contribute to RA initiation and pathogenesis has been a subject of strong debate. Although the RA synovium contains a high number of T cells, the synovial levels of some T cell-derived cytokines can be difficult to detect. A 2017 cancer study, investigating checkpoint inhibitors in patients with various types of cancer, brings direct evidence to this debate[3]. Although checkpoint inhibitors are impressively efficacious in the treatment of some tumours, some of these treated patients developed arthritis and autoimmune diseases[3]. As these drugs function by activating cytotoxic T cells, such immune-related adverse events favour the role of T cells in arthritis induction.

In the field of cancer, the induction of cancer cell death is a key treatment goal. In chronic inflammation, epigenetic changes occur in resident mesenchymal cells (namely synoviocytes); following long periods of exposure to immune-driven inflammation, these cells acquire molecular changes that make them less sensitive to death signals. For example, these cells can acquire mutations in the gene encoding p53, which match the mutations found in inflammation-induced cancer cells[10]. As in cancer, the induction of synoviocyte apoptosis might be of interest for the treatment of arthritis, especially in the later stages of disease when the disease is no longer immune-driven. Such patients usually do not respond to treatments targeting the immune system. In a 2017 preclinical study, transfer of the anti-apoptotic gene *PUMA* to synoviocytes treated with pro-inflammatory cytokines induced apoptosis of these cells *in vitro*; furthermore, intra-articular administration of a *PUMA*-expressing vector to rats decreased joint inflammation and protected against tissue destruction in an experimental model of arthritis[4]. Such an approach might also be used for the treatment of cancer.

In the past, clinical observations in other fields led to the introduction of methotrexate and rituximab as common treatment options for RA. Once again, 2017 has showed us how looking outside the field of arthritis can provide new ideas and valuable insights for RA research.

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The author declares no competing interests.

OSTEOPOROSIS IN 2017

Addressing the crisis in the treatment of osteoporosis

Christian Roux and Karine Briot

A large number of patients with osteoporosis are not receiving appropriate treatment, due in part to concerns regarding drug safety. Great progress has been made to address this crisis in therapy in 2017, including highlighting the patients' views, developing new therapies and treatment strategies and addressing these safety concerns.

The number of fractures related to osteoporosis is expected to increase in the next few decades because of an increase in the number of frail elderly patients at high risk of falls and thus of fractures. Despite the availability of guidelines for the screening of patients at high risk of fracture, and for diagnosing and treating osteoporosis, few patients receive appropriate treatment for osteoporosis, even after a fracture. 2017 saw great strides in addressing this crisis in osteoporosis therapy, including further insight into the view of patients on therapy[1], the unveiling of new information on drug safety[2] and the highlighting of new treatments[3] and treatment strategies[4] (FIG. 1).

The crisis in the treatment of osteoporosis[5] is related to several factors, including the fears and beliefs that patients, as well as physicians[6], hold about the adverse effects of current treatments. Pharmacological treatments for osteoporosis are indicated only for patients with a high risk of fracture. An accurate perception of this risk is crucial for the optimal management of patients. In 2017, Kalluru *et al.*[1] sought to assess the views of patients on their fracture risk and the benefits of osteoporosis treatments. Individuals aged >60 years who were referred for bone density measurements and were not taking any specific osteoporosis treatment were randomized to receive one of four different written and pictorial presentations of their absolute risk of fracture, and the potential benefit they could expect to receive from osteoporosis treatment. The participants regarded a 5-year fracture risk threshold of 50–60% as high enough to consider taking medications to prevent fracture, which is much higher than the recommended risk threshold for commencing therapy. The median 5-year risk initially estimated by the participants was 20% for any fracture (which was higher than the calculated risk). Providing participants with written estimates of fracture risk and treatment benefits led to no or very small changes in the patient's decision concerning treatment, meaning that participants did not believe the estimates they were given, or did not understand the concept of risk. Seeing the results of the bone density scan seemed to reinforce the participants' pre-existing views, regardless of the result. This study[1] confirms previous qualitative studies and demonstrates the contradictions in patients' views about fracture risk, which pose a huge challenge for practitioners.

Concern about the long-term safety of osteoporosis treatments is one of the key reasons for patients either not initiating, or discontinuing, treatments[6]. Given the chronicity of osteoporosis, further information is needed on the long-term effects of such treatments. Clinical trials testing anti-fracture efficacy are normally only placebo controlled for up to 5 years. Extension studies, which observe patients for much longer periods than standard clinical trials, are mandatory for safety assessment, even if efficacy cannot be assessed in such studies owing to the lack of a placebo group.

In this context, 10 years of data on denosumab were provided in 2017 (REF. 2). Denosumab is a fully human monoclonal antibody that binds to RANKL, thereby reducing the number and activity of osteoclasts, resulting in decreased bone resorption. During a 3-year placebo-controlled study[7] of 7,868 women, receiving denosumab treatment (60 mg subcutaneously every 6 months) reduced new vertebral, hip and non-vertebral fractures, compared with placebo. In the extension study published in 2017 (REF. 2), all participants (4,550 women, of whom 2,626 completed follow-up) received open-label denosumab for 7 years, representing a total of 10 years of denosumab treatment for patients who had previously received denosumab (and 7 years for those who had previously received placebo). No new adverse effects were detected during this long-term follow-up period, and the yearly incidence of adverse events of interest (hypocalcaemia, pancreatitis or erysipelas) was as low as that observed in the placebo-controlled trial[7] and stable throughout the extension period. Two cases of atypical femoral fracture (0.8 per 10,000 participant-years) and 13 adjudicated cases of osteonecrosis of the jaw (5.2 per 10,000 participant-years) occurred during follow-up[2]. The annual incidence of new fractures (vertebral: 0.90–1.86%, non vertebral: 0.84–2.55%) remained similar to the incidence observed during the placebo-controlled study[7]; however, there was an increase in bone mineral density in the lumbar spine and total hip (21.7% and 9.2%, respectively) over 10 years[2]. Thus, these data are reassuring for long-term use of this treatment, when appropriate, in fragile patients.

In patients with severe bone deterioration, a rationale exists for considering anabolic therapies rather than antiresorptive therapies. Although only one osteoanabolic therapy (teriparatide) is currently marketed worldwide, abaloparatide is a promising new therapy on the horizon. Daily subcutaneous administration of abaloparatide for 18 months reduced the occurrence of new morphometric vertebral (0.58% versus 4.2%) and non-vertebral (2.7% versus 4.7%) fractures compared with placebo[8]. An extension study is ongoing with open-label alendronate (an antiresorptive therapy); in a 6-month interim analysis, this transitioning strategy (osteoanabolic treatment first, followed by an antiresorptive therapy) was effective in preventing any loss of bone mineral density benefits gained through the initial abaloparatide treatment and was shown to have a persistent anti-fracture effect[9].

Key advances

- The perception osteoporotic patients have of their risk of fracture differs from the measured risk[1]
- Data on the long-term safety of anti-osteoporotic treatments are now available[2]
- New osteoanabolic therapies are now available[3] and a transitioning treatment approach (osteoanabolic treatment first, followed by an antiresorptive therapy) seems to be an effective novel strategy[4]

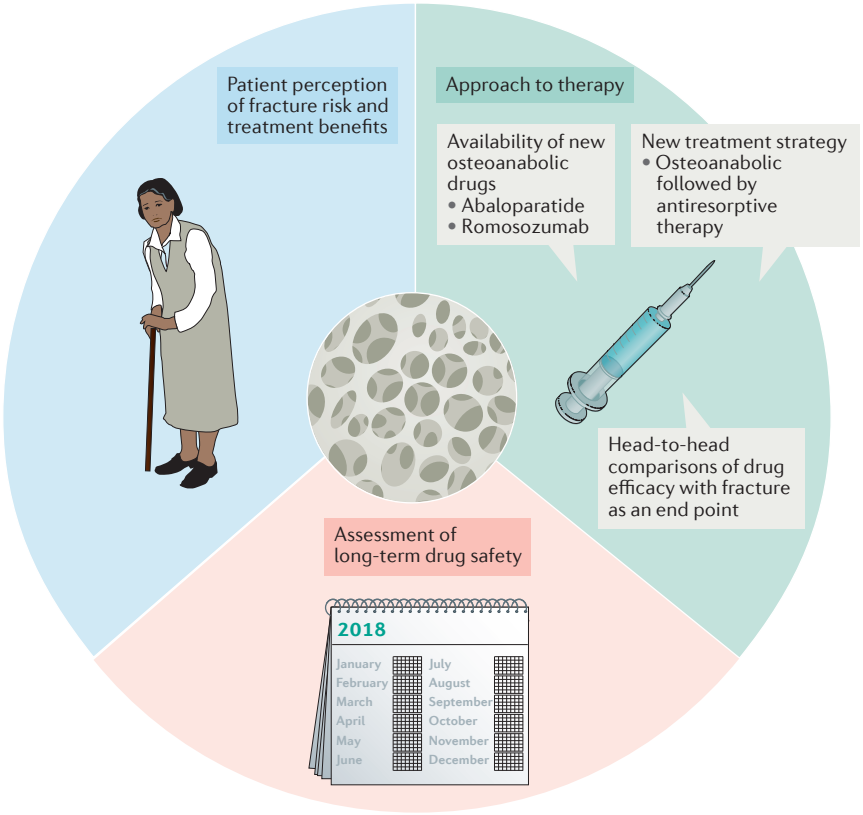

Figure 1 | **Advances in osteoporosis in 2017.** Multiple advances have been made in osteoporosis therapy in 2017, including new insights into the patient perception of fracture risk and treatment benefits, assessment of the long-term safety of drugs with an already proven efficacy and key advances in therapeutic approaches.

In 2017, two studies[3,4] were published that change the paradigm of clinical studies in osteoporosis; both studies are head-to-head trials (that is, they compare two treatments that have a proven anti-fracture efficacy), and both have adequate power to consider fractures as a primary end point.

Kendler *et al.*[4] compared the effectiveness of teriparatide (20 µg subcutaneous administration once daily) and the antiresorptive drug risedronate (35 mg oral administration once weekly) over 2 years in a randomized study involving 1,360 postmenopausal women with prevalent vertebral fractures and low bone density. Over 2 years, patients receiving risedronate had an increased incidence of new vertebral fractures compared with patients receiving teriparatide (12% and 5.4%, respectively), although the incidence of non-vertebral fractures was similar between the two groups. Adverse events were similar in the two groups, and no cases of osteonecrosis of the jaw or atypical femoral fracture were reported.

Romozumab is a monoclonal antibody that inhibits sclerostin, an osteocyte product that inhibits osteoblast activity and bone formation. Romosozumab treatment demonstrated beneficial effects on bone mineral density and in preventing vertebral fracture in a placebo-controlled study of postmenopausal women with osteoporosis[10]. In 2017, Saag *et al.*[3] compared the effectiveness of two strategies: romosozumab therapy (210 mg subcutaneous once monthly; romosozumab group) or alendronate therapy (oral 70 mg once weekly; alendronate group) for 1 year (in a blinded fashion) followed by open-label alendronate in both groups, in postmenopausal women (74 years of age on average) with a high risk of fracture. The incidence of new vertebral and non-vertebral fractures was lower in the romosozumab group (6.2% and 8.7%, respectively) than in the alendronate group (11.9% and 10.6%, respectively). Bone mineral density increased by 15% and 7% at the spine and hip, respectively. During the open label period, two occurrences of osteonecrosis of the jaw (one in each group) and six occurrences of atypical femoral fractures (two and four, respectively, in the romosozumab and alendronate groups) were recorded. An imbalance in adjudicated serious cardiovascular events was observed during the first year: 2.5% and 1.9% in the romosozumab and alendronate groups, respectively[3]. The involvement of sclerostin in vascular calcification might explain the apparent cardiovascular effects of romosozumab. However, such an imbalance was not reported in the previous placebo-controlled study of romosozumab[10]; thus the cause of the imbalance in the current study needs further evaluation.

These two therapeutic trials represent fundamental advances in the field of osteoporosis. They are the first trials to suggest that we can go away from the 'one treatment fits all' approach (one bisphosphonate for every patient) to an individualized approach (an anabolic followed by an antiresorptive therapy). Careful selection of patients at high risk of fracture and selection of the best therapeutic strategy are strongly recommended, as the prevention of fragility fractures is within our reach.

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PAEDIATRIC RHEUMATOLOGY IN 2017

Child-centred research is the key to progress

Michael W. Beresford and Athimalaipet V. Ramanan

The rarity, severity and complexity of paediatric rheumatic diseases make progress in treating these diseases a challenge. In 2017, a new series of recommendations for treatment, studies that unravel the complexity of juvenile idiopathic arthritis and clinical trials that tackle sight-threatening uveitis have helped to improve paediatric care.

Paediatric rheumatic diseases prove challenging to clinicians and scientists seeking to improve standards of care and to ultimately cure these potentially devastating childhood disorders. The key to advances in understanding and treating these diseases has been to keep the child as the focal point of collaborative, multidisciplinary initiatives (FIG. 1). Indeed, despite paediatric rheumatology being one of the last paediatric subspecialities to develop, the rapid progress that has been made in translational research, clinical science and patient engagement is proving exemplary across paediatric specialties. In 2017, advances have been made in improving the health and well-being of children and young people with a range of systemic autoimmune and autoinflammatory diseases[1], in understanding the complexity of juvenile idiopathic arthritis (JIA)[2] and in treating comorbidities associated with JIA, including uveitis[3].

All children have a right to take part in clinical research to improve our knowledge and understanding of paediatric diseases. The Single Hub and Access Point for Paediatric Rheumatology in Europe (SHARE) initiative was launched in 2012 and aimed to optimize and disseminate diagnostic and management regimens for children and adolescents with rheumatic diseases. In 2017, SHARE's key recommendations for collaborative paediatric research, including recommendations for biobanking, set a robust international framework for the implementation of research across and beyond international borders[1].

This international framework, the first of its kind, is underpinned by a structured and comprehensive evidence-based review process and importantly, integrates the perspectives of the families of children and young people living with paediatric rheumatic diseases. Although formulated within a given geographical region and specific for paediatric rheumatic disorders, the approach of the SHARE initiative is transferrable to other collaborative research projects in rare paediatric diseases. In 2017, four evidence-based or consensus-based sets of SHARE recommendations were published. These recommendations set important minimal 'standards of care' for the diagnosis and treatment of juvenile dermatomyositis[4], paediatric antiphospholipid syndrome[5], childhood-onset systemic lupus erythematosus[6] and childhood lupus nephritis[7]. Together, these recommendations help to harmonize and optimize care and research to produce the best outcomes for all patients.

Systemic JIA (sJIA) has long been recognized as different from other types of JIA in its clinical manifestations and the response of patients to medications such as methotrexate and anti-TNF therapies. Of all the JIA subtypes, sJIA is associated with the greatest degree of disease-related morbidity. Although deaths from JIA are rare, most are caused by macrophage activation syndrome, a complication seen mainly in children with sJIA. In general, sJIA is considered to be an autoinflammatory condition that is driven by the innate immune system.

In 2017, Ombrello *et al.*[2] provided further evidence supporting this hypothesis. The International Childhood Arthritis Genetics (INCHARGE) consortium gathered data on 982 children from nine countries in three continents[2], exemplifying the great spirit of international collaboration within paediatric rheumatology. Using this cohort, Ombrello *et al.*[2] performed a genome-wide association study on 770 children with sJIA and identified two loci that exceeded the threshold for genome-wide significance ($P < 2.5 \times 10^{-8}$). In addition to the previously recognized MHC locus, the authors also identified a novel locus on the short arm of chromosome 1 that includes 14 sJIA-associated single nucleotide polymorphisms (SNPs) that span 20.6 kb (REF. 2). A further 23 novel loci were also putatively associated with sJIA ($P < 5 \times 10^{-8}$). Most importantly, none of the key loci identified intersected with susceptibility loci for other types of JIA[8]. These findings add weight to the feeling held among clinicians that sJIA is both clinically and genetically distinct from other types of JIA. The priority now will be to explore the functional significance of these loci and to identify therapeutic novel targets to specifically treat children with sJIA, which despite recent advances is still associated with a substantial degree of morbidity and mortality.

Children with JIA, particularly young children with mild forms of arthritis (such as oligoarthritis), are at high risk of developing uveitis. JIA-associated uveitis is associated with a large degree of morbidity. Almost half of those with JIA-associated uveitis develop visual impairment that leads to cataracts, glaucoma and loss of vision. Although there have been many clinical trials of novel biologic agents in JIA over the past few years, they have all specifically excluded children with active uveitis.

In 2017, the paediatric rheumatology community worked closely with parents and patients to develop a trial that sought to definitively answer the crucial question of whether anti-TNF therapy in children with methotrexate-refractory JIA-associated uveitis is effective[3]. In developing the SYCAMORE trial[3], the randomized placebo phase withdrawal design adopted by the majority of JIA trials was deemed to be unsatisfactory by both patients and clinicians. Working closely with patients' families, a pragmatic but robust 2:1 randomized placebo-controlled trial was designed. This design ensured rigorous trial methodology and appropriate safeguards and 'escape routes' for children with active, uncontrolled disease.

Key advances

- Key recommendations set a robust international framework for collaborative research that crosses borders and ensures equity in access to care for the diagnosis and treatment of rare childhood rheumatic diseases[1]
- Systemic juvenile idiopathic arthritis (JIA) is clinically and genetically distinct from other types of JIA[2]
- Adalimumab with methotrexate is an effective treatment for children with JIA-associated uveitis[3]

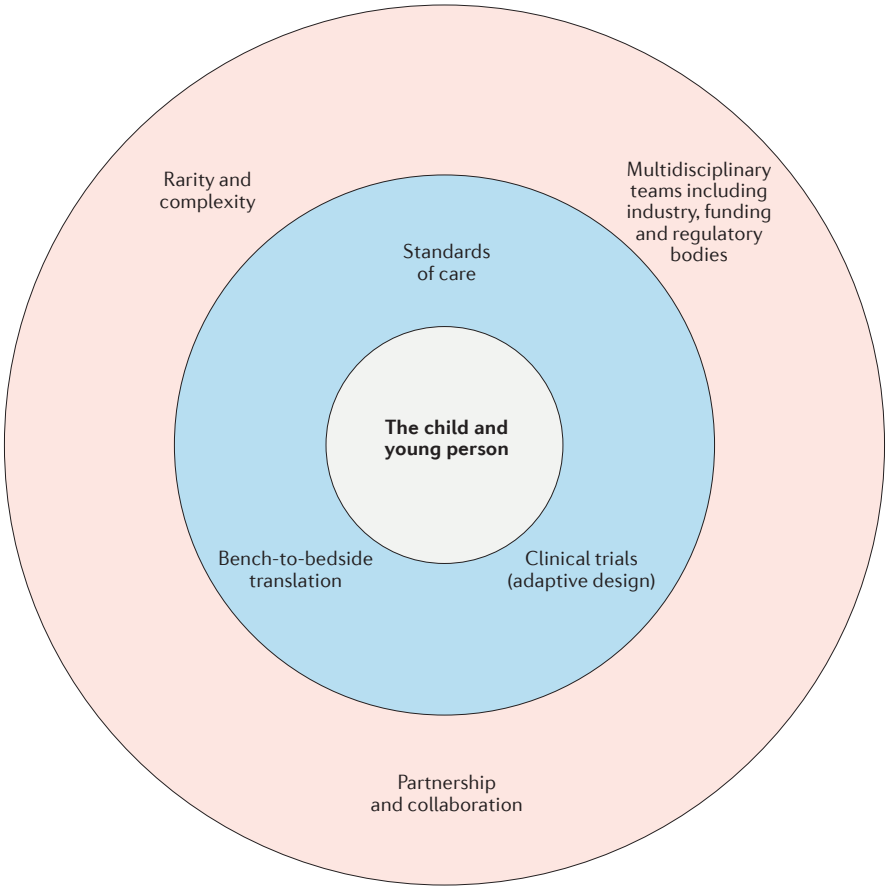

Figure 1 | **Keeping the child at the centre of paediatric research.** Key advances from 2017 are highlighted in the blue ring and major challenges that remain are shown in the red ring. To make progress in tackling rare and complex disorders that affect children and young people, we need to foster partnerships between multidisciplinary teams and collaborations with health-care and research funding bodies, industry and regulators to improve clinical trials, bench-to-bedside translation and standards of care.

The SYCAMORE trial was stopped early after recruitment of 90 patients (rather than the desired 114) by the Independent Data and Safety Monitoring Committee and demonstrated highly significant results ($P < 0.0001$) in favour of treating uveitis with adalimumab and methotrexate compared with treating with methotrexate alone[3].

The SYCAMORE trial excluded children with idiopathic uveitis; however, it is now widely acknowledged that children with idiopathic chronic anterior uveitis have essentially the same disease as children with JIA-associated uveitis[9]. The use of placebo meant that children who are intolerant of methotrexate (~30–40% of all children with JIA-associated uveitis) were not included in the SYCAMORE trial[9], making any conclusions about the role of adalimumab as a monotherapy in the management of JIA-associated uveitis difficult. However, this trial demonstrated that proactive engagement with patients and parents can successfully lead to clinical trials of novel agents that use methodologically robust designs and that can actually answer clinically important questions.

The success of the SYCAMORE trial confronts the position accepted by many that it is not feasible or possible to carry out placebo-controlled studies in children. The SYCAMORE trial is a paradigm for partnership between clinicians, the families of children and young people affected by rheumatic diseases, government agencies and charities in studying rare diseases. Clinicians need to be able to directly address questions raised by children and parents such as 'Which drug best works for me?'. To achieve this goal, researchers need to step away from conventional study designs and to include adaptive designs or head-to-head studies of new agents versus existing approved biologics[10]. The clinical community, children, families, industry and regulators need to work together to put the child at the centre of paediatric research and to ensure that trials are not just meeting regulatory requirements but are answering life-changing questions.

The advances discussed above build on two decades of progress in our understanding of the mechanisms that underlie rheumatic diseases, an explosion of new therapies underpinned by paediatric clinical trials and the introduction of new biologic agents to the routine armoury of medications used by clinicians. But progress requires continued commitment. The international community is encouraged to gather around and seek constructive ways to overcome the national, international, institutional, clinical and academic barriers that can limit progress.

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RHEUMATOID ARTHRITIS IN 2017

Protective dietary and hormonal factors brought to light

Jeffrey A. Sparks and Karen H. Costenbader

Tremendous progress has been made in the identification of rheumatoid arthritis (RA) risk factors in 2017. The results of epidemiological studies highlighted dietary and hormonal factors that are associated with slowing the transition from one preclinical phase of RA to another, potentially protecting individuals from developing RA.

The development of anti-citrullinated protein antibodies (ACPAs) and the subsequent onset of ACPA$^+$ rheumatoid arthritis (RA) are central to the pathogenesis of seropositive RA. Some individuals who have an increased genetic susceptibility to RA will develop ACPAs, of whom a proportion will go on to present symptomatically with arthralgia and early inflammatory arthritis, before being diagnosed with ACPA$^+$ RA[1]. A growing list of environmental factors, including cigarette smoke, inhaled particulates (such as silica), diet, hormones, medication use and infections can affect the transitions between these preclinical phases in the pathogenesis of RA[2] (FIG. 1). Previous research has identified factors, in particular smoking, that accelerate these transitions and increase the risk of developing ACPA$^+$ RA[2]. Although the identification of smoking as an ACPA$^+$ RA risk factor is of crucial importance, most patients with RA are non-smokers, and the incidence of RA seems to be stable despite a decrease in the number of individuals who smoke. Other environmental factors are therefore likely to be important contributors to RA risk. The epidemiological observations that consumption of omega-3 fatty acids[3], a long-term healthy diet[4] and use of oral contraceptives[5] might protect against RA offer potential strategies to lower RA risk and to generate hypotheses concerning the biological mechanisms of these environmental factors.

In 2017, several studies have established the role of dietary factors in the pathogenesis of RA. Gan *et al.*[3] investigated whether omega-3 fatty acids are important in the preclinical phases of RA on the basis of previous studies that associated fish[6] or fish oil[7] intake with a decreased risk of RA and favourable outcomes among patients with RA. Gan *et al.*[3] measured the composition of omega-3 fatty acids in erythrocyte membranes from 47 ACPA$^+$ individuals who did not have diagnosed RA, 10 of whom had undifferentiated inflammatory arthritis.

> “these results provide further evidence that diet is an important risk factor for RA”

High levels of total erythrocyte-bound omega-3 fatty acids were strongly associated with a reduced risk of having undifferentiated inflammatory arthritis (OR 0.09, 95% CI 0.01–0.85 per increasing standard deviation)[3]. The authors then followed 35 ACPA$^+$ individuals who did not have inflammatory arthritis and found that high levels of docosapentaenoic acid at baseline protected against the development of inflammatory arthritis (HR 0.52, 95% CI 0.27–0.98) during a mean follow-up of 2.6 years[3].

In addition to providing new insights into RA pathogenesis, these results offer ACPA$^+$ individuals at high risk of developing RA a dietary modification that might help to lower that risk. These findings complement those of a previous study that showed that increasing levels of erythrocyte-bound total omega-3 fatty acids lowered the risk of ACPA positivity (OR 0.44, 95% CI 0.21–0.93) in 136 unaffected individuals at high risk of RA due to seropositivity or positivity for the HLA shared epitope[3]. Together, these studies demonstrate that omega-3 fatty acids might have dual actions: lowering the risk of developing ACPAs and preventing the onset of inflammatory arthritis once ACPAs are present. By extension, these studies suggest that omega-3 fatty acids might decrease RA risk, although this hypothesis has yet to be proved. These small but provocative studies provide the rationale for prospective studies and clinical trials of omega-3 fatty acids for the prevention of RA.

In another dietary study, Hu *et al.*[4] examined whether dietary quality was associated with the risk of RA in American women using the Alternative Healthy Eating Index (AHEI) and the prospective Nurses’ Health Studies cohorts. The AHEI was developed using expert opinion of foods and nutrients related to the risk of chronic diseases[8]. The AHEI uses 11 food and nutrient categories that cover healthy foods (fruits, vegetables, whole grains, nuts, omega-3 fatty acids, polyunsaturated fatty acids and moderate amounts of alcohol) and unhealthy foods (sugar-sweetened beverages, red or processed meat, *trans* fats and sodium) to calculate an overall score[8]. Hu *et al.*[4] analysed the long-term quality of the participants’ diets as a cumulative average AHEI score using repeated food frequency questionnaires, and categorized women into AHEI quartiles. Those in the highest AHEI quartile were considered to have the highest dietary quality and those in the lowest AHEI quartile were considered to have the lowest dietary quality. In all, data from 169,989 women were analysed, of whom 1,007 developed RA during the 3.7 million person-years of follow-up[4]. Among women ≤55 years of age, the highest, and therefore healthiest, AHEI quartile had a reduced HR of 0.67 (95% CI 0.51–0.88)

Key advances

- High levels of erythrocyte-bound omega-3 fatty acids are associated with decreased progression to inflammatory arthritis among anti-citrullinated protein antibody-positive individuals who do not have rheumatoid arthritis (RA)[3]
- Compared with an unhealthy diet, long-term adherence to a healthy diet was inversely related to the risk of developing RA in individuals of 55 years of age or younger[4]
- Women who had ever used oral contraceptives had a lower risk of developing RA compared with those who had never used oral contraceptives[5]

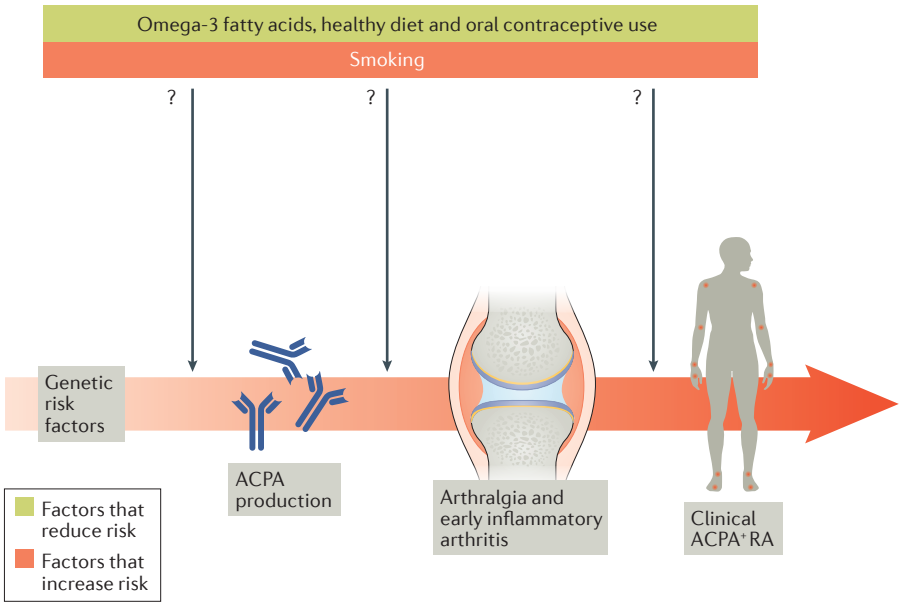

Figure 1 | **Preclinical phases of ACPA⁺ rheumatoid arthritis.** Individuals at increased genetic risk of rheumatoid arthritis (RA) can develop anti-citrullinated protein antibodies (ACPAs). Environmental factors such as smoking increase the risk of ACPA production, whereas other environmental factors, such as omega-3 fatty acid intake, might decrease the risk of ACPA production. ACPA⁺ individuals might then go on to develop arthralgias and undifferentiated inflammatory arthritis, which can progress to clinically apparent ACPA⁺ RA. Similar to the risk of ACPA production, smoking increases the risk of progression to inflammatory arthritis, whereas intake of omega-3 fatty acids might decrease this risk. A healthy diet and use of oral contraceptives might decrease the overall risk of RA, but the mechanisms and particular preclinical phases of RA affected by these risk factors are unclear.

for RA compared with those with the lowest dietary quality ($P = 0.002$ for trends across quartiles)[4]. This protective effect was most pronounced for seropositive RA among those aged ≤55 years (HR 0.60, 95% CI 0.42–0.86, $P = 0.003$ for trend)[4]. However, the healthiest diet by AHEI score was not associated with RA among those >55 years of age[4]. Two AHEI components were particularly associated with a reduced risk of RA among those ≤55 years of age[4]: reduced intake of red or processed meat (HR 0.58, 95% CI 0.43–0.79) and moderate alcohol intake (HR 0.67, 95% CI 0.51–0.89)[4]. However, intake of omega-3 fatty acids was not associated with RA risk in this study[4]. Overall, these results provide further evidence that diet is an important risk factor for RA, and provide insight into potential dietary guidance for individuals at risk of developing RA.

The hormonal and metabolic changes that occur during menopause might explain why diet was only associated with RA that develops before 55 years of age[4], but this association has not been firmly established[9]. However, hormones themselves might be associated with the risk of RA.

In 2017, Orellana *et al.*[5] investigated the associations between oral contraceptive use and RA risk in women in a large Swedish case–control study. Women who had ever used oral contraceptives had a modestly decreased risk of RA (OR 0.87, 95% CI 0.78–0.97) compared with those who had never used oral contraceptives[5]. This protective effect was present for ACPA⁺ RA (OR 0.84, 95% CI 0.74–0.95), but not for ACPA⁻ RA[5]. A long duration (≥7 years) of oral contraceptive use further lowered the risk of developing ACPA⁺ RA (OR 0.80, 95% CI 0.69–0.93)[5]. Ever smokers who had never used oral contraceptives had a particularly increased risk of developing ACPA⁺ RA (OR 2.34, 95% CI 1.95–2.82) compared with those who had never smoked but had used oral contraceptives[5]. The hormonal components and doses of the oral contraceptives used varied during the study period, so the importance of particular hormones and their relative doses for RA risk are unclear[5]. Given the study design, it is uncertain during which phase of RA development hormones exert their protective effect. However, this study provides further evidence that hormones are important in the pathogenesis of ACPA⁺ RA, which is of particular interest given the predominance of RA in women and the differences in risk related to age and menopause[4,9].

The development of RA is complex and is likely to differ between individuals, but the epidemiological advances made in 2017 elucidate factors that potentially protect against RA. These findings provide a rationale for RA prevention strategies using dietary and hormonal interventions. We are now closer to understanding the natural history of RA and how we might best intervene to delay or even prevent RA onset in the near future.

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Competing interests statement
The authors declare no competing interests.

OSTEOARTHRITIS IN 2017

Latest advances in the management of knee OA

Timothy E. McAlindon and Raveendhara R. Bannuru

Osteoarthritis research in 2017 provided new insights into the long-term effects of intra-articular glucocorticoids, and also led to the approval of a novel, longer-lasting glucocorticoid formulation. New drugs for the treatment of osteoarthritis also emerged this year, including a small-molecule inhibitor of the Wnt signalling pathway.

In the past few years, interesting advances have occurred in the field of osteoarthritis (OA) research — most notably in the development of sophisticated drug-delivery systems as well as new therapeutic agents. Developments in 2017 included the approval by the FDA of an extended-release glucocorticoid formulation that uses a novel drug delivery system[1], and the publication of a randomized controlled trial (RCT) describing the consequences of long-term use of conventional intra-articular glucocorticoids in the treatment of OA[2]. Another RCT published within the past year suggested that a small-molecule inhibitor of the Wnt signalling pathway, SM04690, could have disease-modifying effects in OA, prompting a call for further clinical trials[3] (FIG. 1).

Glucocorticoids are one of the most widely recommended treatments for knee OA, but clinical trials have shown that their analgesic effect declines within 2–4 weeks[4]. To overcome this limitation, new delivery systems such as poly(lactic-co-glycolic acid) (PLGA) microspheres have been developed in an attempt to prolong the action of these drugs. FX006 is an extended-release formulation of triamcinolone acetonide in PLGA microspheres, designed to maintain the concentration of this synthetic glucocorticoid in the joint for several months after a single dose. In a multicentre phase II randomized double-blind dose-finding study published in 2015 (REF. 5), a single intra-articular injection of 40 mg of FX006 performed comparably to 40 mg of immediate-release triamcinolone acetonide and provided greater pain relief from weeks 5–10 post-treatment. A follow-up phase IIb study published in 2017, involving 306 patients with knee OA[6], showed that 32 mg of FX006 provided significant improvements in pain relief compared with placebo from weeks 1–11 and at week 13; however, the study failed to achieve its primary end point of a significant difference between FX006 and placebo at 12 weeks. In a subsequent phase III study of 484 patients with knee OA[1], conducted in support of the FDA pre-market approval application, FX006 demonstrated a statistically significant reduction in pain intensity versus placebo at 12 weeks, although this reduction was not statistically significantly different from that achieved with immediate-release triamcinolone acetonide. On the basis of this last study, FX006 was approved by the FDA for the management of knee OA. The active component drug is not new to the field, but the approval of FX006 represents a technological advance that provides a model for targeted delivery approaches to treating OA.

Although the short-term analgesic effects of glucocorticoids for knee OA are well-established, the benefits of their long-term use are questionable. A clinical trial published in 2017 examined the effects of intra-articular triamcinolone acetonide injected every 3 months over the course of 2 years on knee pain and the progression of cartilage loss in patients with OA[2]. In this study, 140 patients with knee OA with features of synovitis on ultrasonography were randomly allocated to receive either triamcinolone acetonide or placebo. This study demonstrated a significantly greater loss in cartilage volume in the triamcinolone acetonide group than in the placebo group, with no significant difference in pain reduction between the two groups. Whereas negative anabolic effects of glucocorticoids have been documented in many laboratory studies, the absence of a long-term benefit for pain is perhaps surprising given the short-term effects on knee pain that have been observed in numerous clinical trials. The absolute increase in cartilage loss observed in the patients treated with triamcinolone acetonide translates to approximately 2% per year. This amount of cartilage loss is of concern, because each 1% increase in the rate of tibial cartilage loss corresponds to a 20% increased risk of undergoing total knee arthroplasty within 4 years[7]. Another study showed that the degree of joint space narrowing was positively associated with subsequent total knee arthroplasty within 15 years (OR 5.0, 95% CI 2.6–9.9)[8]. Although the glucocorticoid-treated group did not have worse clinical outcomes than the placebo group in the trial, the observation of

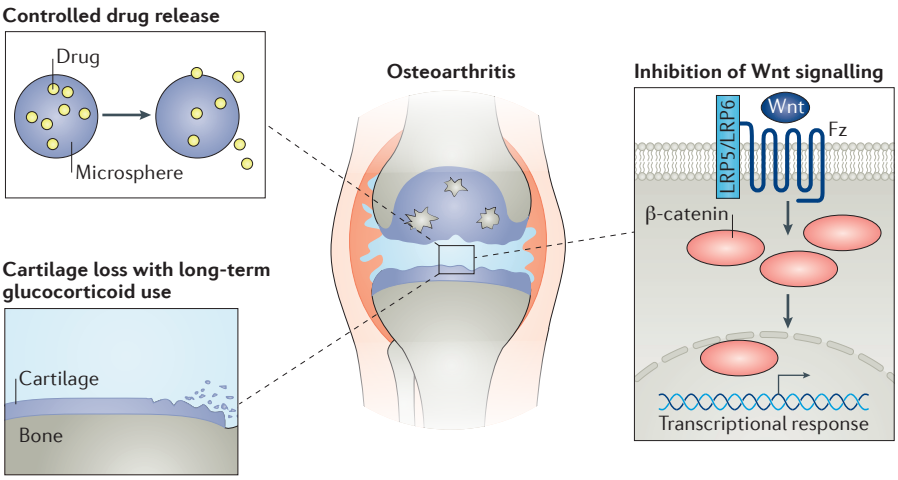

Figure 1 | **Research advances in osteoarthritis management.** A novel drug-delivery system can prolong the analgesic effect of intra-articular glucocorticoids for knee osteoarthritis (OA). Results of clinical trials shed light on the long-term effects of intra-articular glucocorticoids and on the therapeutic potential of a small-molecule inhibitor of the Wnt signalling pathway for OA. Fz, Frizzled; LRP, lipoprotein receptor-related protein.

Key advances

- FX006, an extended-release triamcinolone acetonide, was approved by the FDA for the management of knee osteoarthritis (OA) pain[1]
- A clinical trial demonstrated that treatment with intra-articular glucocorticoids for 2 years resulted in greater loss in cartilage volume than placebo in patients with symptomatic knee OA[2]
- SM04690, a small-molecule inhibitor of the Wnt signalling pathway, showed promise in initial clinical trials, in which it controlled clinical symptoms, preserved joint space width and was well-tolerated[3]

increased cartilage loss should be integrated into risk–benefit discussions with individual patients, as other work has shown that increased rates of cartilage loss predict the long-term need for arthroplasty.

OA is characterized by degradation of cartilage and thickening of subchondral bone. The Wnt signalling pathway stimulates the production of catabolic proteases that have been implicated in matrix degradation and modulates the differentiation of osteoblasts and chondrocytes. Animal models of OA and *in vivo* studies have provided evidence that the Wnt pathway inhibitor SM04690 might cause cartilage regeneration and provide protection from cartilage catabolism[9]. A 24-week phase I study published in 2017 assessed the pharmacokinetics, safety and exploratory efficacy of intra-articular SM04690 in moderate to severe knee OA[3]. This multi-centre RCT, which enrolled 61 patients with knee OA (50 of whom received SM04690 and 11 placebo), demonstrated that SM04690 was safe and well tolerated, and no significant differences in bone mineral density were observed between the two groups. Patients who received SM04690 showed improvements in all exploratory efficacy measures — including pain, function and OMERACT–OARSI response measures — from baseline to 24 weeks. Additionally, a significant improvement in joint space width was observed in the SM04690 group versus the placebo group ($P=0.02$). The results from a completed phase II trial are expected soon. Given that the effect of intra-articular saline placebo is about 1.6 times that of acetaminophen[10], it is noteworthy that these intra-articular treatments (FX006 and SM04690) have demonstrated a significantly better effect than intra-articular saline placebo.

Although the OA research community has made exciting progress in the management of knee OA over the past year, these innovations are still emerging. More extensive clinical trial data exploring the benefits and harms of repeated injections of FX006 are necessary to develop a comprehensive safety and efficacy profile for this treatment, particularly in light of new data on the long-term effects of glucocorticoid use. Likewise, further research in the form of larger phase II trials are required to fully examine the effects of SM04690.

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T.E.M. declares that he has acted as a consultant for Flexion Therapeutics and as an Advisory Board member for Samumed. R.R.B. declares no competing interests.

BLADDER CANCER IN 2017

Advancing care through genomics and immune checkpoint blockade

Matthew D. Galsky

Landmark papers published in 2017 have advanced our understanding of the molecular heterogeneity of urothelial cancer, provided insights into the genomic evolution of the disease in the context of metastasis and therapy, and established new treatment standards for patients with previously limited treatment options.

Urothelial cancer of the bladder is a relatively common malignancy ranking approximately ninth among the most commonly diagnosed cancers worldwide. Nevertheless, treatment advances for bladder cancer have generally lagged behind those for other malignancies. Several potential reasons exist for the historically slow pace of progress: the most prominent barriers have been a poor understanding of disease biology, a paucity of prospective clinical trials, and a disconnect between interventions supported by level 1 evidence and implementation of those interventions in clinical practice (FIG. 1). Exemplified by key publications from 2017 that address each of these barriers, the wheels of progress to improve the treatment for patients with bladder cancer are now clearly turning.

High-throughput genomic interrogation technologies for analysis of clinical specimens have enabled an unprecedented understanding of the molecular complexity and heterogeneity of urothelial cancer of the bladder. The Cancer Genome Atlas (TCGA) has arguably provided the most expansive and complete profile of urothelial cancer genomics[1]. In 2017, Robertson *et al.*[2] reported the second comprehensive analysis of the urothelial cancer cohort of TCGA, which has now been expanded to 412 specimens of muscle-invasive bladder cancer (MIBC). Similar to the initial analysis, crucial observations were made with the potential to affect clinical care in the near future. To better understand the basis for the high mutation burden of urothelial cancer, the researchers performed Bayesian non-negative matrix factorization and identified five mutation signatures. Remarkably, APOBEC-mediated mutagenesis was responsible for most of the single-nucleotide variants (SNVs), and a subset of patients with both high levels of APOBEC signature mutagenesis and high mutation burden had a relatively favourable 75% 5-year survival probability. In addition to findings from the first TCGA report[1], a further 34 frequently mutated genes were identified; these included alterations with potential therapeutic implications, for example in *ATM* and *ERBB2*, which were mutated in 14% and 12% of the 412 samples, respectively[2]. Unbiased consensus clustering of RNA sequencing data identified five distinct mRNA expression subtypes: luminal-papillary (35%), luminal-infiltrated (19%), luminal (6%), basal-squamous (35%), and neuronal (5%). Interestingly, the neuronal subset was associated with particularly poor outcomes and often lacked histological evidence of neuroendocrine differentiation.

Based on integration of the RNA and DNA sequencing results, Robertson *et al.*[2] proposed an expression-based, subtype-stratified approach to MIBC treatment for prospective testing in clinical trials. For luminal-papillary subtypes, fibroblast growth factor receptor 3 (FGFR3) inhibition was proposed, owing to enrichment with *FGFR3* alterations, putative resistance to neoadjuvant chemotherapy, and the generally favourable outcomes of patients with these tumours. For luminal-infiltrated subtypes, immune checkpoint blockade was proposed, owing to the presence of immune cell infiltration, medium expression levels of programmed cell death 1 ligand 1 (PDL1), findings from previous clinical studies that correlated the original TCGA cluster II (which is now primarily encompassed by the luminal-infiltrated subtype) with response to atezolizumab[3], and a putative low likelihood of response to neoadjuvant chemotherapy. For basal-squamous subtypes, neoadjuvant chemotherapy or immune checkpoint blockade was proposed, owing to infiltration of immune cells, high PDL1 expression, and a putative benefit from neoadjuvant chemotherapy. For the neuronal subtype, etoposide plus cisplatin-based neoadjuvant chemotherapy was proposed, extrapolating from the treatment of small cell cancers arising from other anatomical sites. This framework has the potential to advance the application of molecular medicine in MIBC, but clinical validation is still required.

The large-scale efforts of TCGA are centred on defining the genomic landscape of untreated primary tumours, but such specimens provide limited insight into tumour evolution and mechanisms of acquired therapeutic resistance. Seeking to understand the degree of clonal divergence between primary and metastatic urothelial cancer, and to explore how chemotherapy influences genomic evolution, Faltas and colleagues[4] performed whole-exome sequencing of 72 urothelial tumours from 23 patients, including 16 matched pairs of primary and metastatic specimens. The researchers made several important observations. First, no statistically

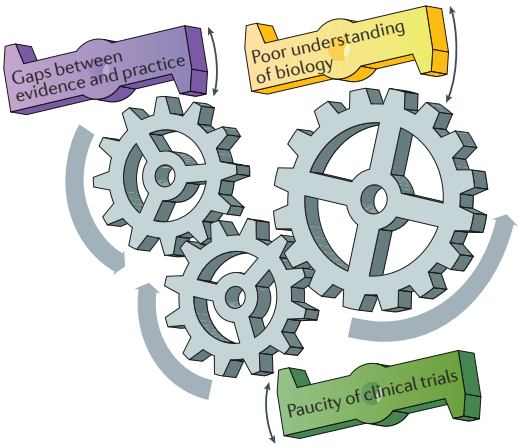

Figure 1 | **Key barriers to progress in bladder cancer therapy.** Treatment advances for bladder cancer have historically lagged behind those for other malignancies owing to several barriers. Key publications from 2017 set the wheels of progress in motion to improve the treatment for patients with bladder cancer.

Key advances

- Comprehensive molecular profiling of 412 muscle-invasive bladder tumours revealed new molecular classifications of this disease associated with distinct outcomes and potential therapeutic implications[1]
- Molecular profiling of paired primary and metastatic urothelial tumours, as well as samples taken before and after chemotherapy, demonstrated early branching genomic evolution with implications for the development of targeted therapies[2]
- The programmed cell death protein 1 (PD1) antibody pembrolizumab improves response rate and survival and has a favourable toxicity profile compared with standard chemotherapy in patients with metastatic urothelial cancer that is progressing despite previous platinum-based chemotherapy[3]
- The PD1 antibody pembrolizumab and the PDL1 antibody atezolizumab demonstrated encouraging durable anticancer activity in a subset of cisplatin-ineligible chemotherapy-naive patients with metastatic urothelial cancer[4,5]

significant difference in the number of SNVs was demonstrated between prechemotherapy and postchemotherapy tumours. These findings challenge the hypothesis that conventional chemotherapy enhances subsequent response to immune checkpoint blockade as a result of an increased mutational (and neoantigen) burden. Second, only 28% of mutations were shared by pretreatment and post-treatment tumours; even mutations in driver genes were not consistently shared in paired specimens. These findings have implications for the clinical development of therapies that target specific molecular alterations and highlight the potential importance of performing biopsies on metastatic tumours when screening patients for their suitability for such treatment strategies. Third, L1CAM and integrin signalling were identified as potential mediators of cisplatin resistance in urothelial cancer. Finally, in reconstructed evolutionary trees, the primary tumour was generally positioned as a branch rather than the trunk, indicating parallel evolution of multiple cell populations during early stages of tumour development.

Since 2014, at least five distinct antibodies to PD1 and PDL1 have demonstrated antitumour activity in metastatic urothelial cancer leading to their integration into standard care[3,5,6]. Most of the data supporting the use of PD1 or PDL1 blockade is derived from single-arm phase I or II studies offering a limited understanding of the relative efficacy and safety of these agents in the context of conventional chemotherapy. In 2017, Bellmunt and colleagues[5] reported the results of the phase III trial Keynote-045, in which 542 patients with metastatic urothelial cancer that had progressed despite previous platinum-based chemotherapy were randomized to receive either the PD1 antibody pembrolizumab or chemotherapy (paclitaxel, docetaxel, or vinflunine). Pembrolizumab treatment compared with chemotherapy was associated with a significant improvement in objective response rate (21.1% versus 11.4%) and overall survival (median 10.3 months versus 7.4 months; HR for death 0.73, 95% CI 0.59–0.91; $P=0.002$). No significant difference was observed in progression-free survival. In addition, the benefit of treatment with pembrolizumab was not limited to patients with increased PDL1 expression measured in archival tumour specimens. Importantly, this trial confirmed that responses to PD1 blockade and chemotherapy are not only quantitatively but also qualitatively different. In the pembrolizumab group, 68% of patients had an estimated duration of response of ≥12 months compared with 35% in the chemotherapy group. Pembrolizumab was also better tolerated than chemotherapy: 49.4% of patients developed a grade 3–5 adverse event with chemotherapy compared with 15% of patients treated with pembrolizumab. Immune-related adverse effects were more common in patients randomized to pembrolizumab, but the incidence of individual any-grade events was low: thyroid abnormalities in 9.4%, pneumonitis in 4.1%, colitis in 2.3%, and adrenal insufficiency in 0.4% of patients.

Cisplatin-based combination chemotherapy has been the standard treatment for metastatic urothelial cancer for several decades. However, a large subset of patients are suboptimal candidates for cisplatin-based chemotherapy owing to poor renal function and/or other comorbidities[7]. Carboplatin-based regimens have been used in this population, but these regimens were associated with generally suboptimal outcomes and considerable treatment-related toxicity. Two large single-arm phase II trials reported in 2017 explored PD1 or PDL1 blockade in this patient population leading to regulatory approval of these agents[8,9]. In Keynote-052, 370 cisplatin-ineligible patients were treated with pembrolizumab, leading to an objective response rate of 24% (95% CI 20–29%)[8]. In IMvigor 210, 123 cisplatin-ineligible patients received the PDL1 antibody atezolizumab, leading to an objective response rate of 23% (95% CI 16–31%)[9]. Both studies demonstrated that most responses were durable with median durations of response not reached, and the adverse event profile was similar to other studies of PD1 or PDL1 blockade. Randomized trials comparing first-line PD1 and/or PDL1 blockade with chemotherapy are pending, but the results of Keynote-052 and IMvigor 210 have already had substantial effects on clinical practice, bridging the efficacy–effectiveness gap encountered with many treatments for metastatic urothelial cancer. PD1 and PDL1 blockade not only leads to durable responses in a subset of patients in trial settings but can also be applied broadly to patients in routine practice owing to the relatively low likelihood of severe adverse events with this approach.

Results published in 2017 have provided major insights into the molecular heterogeneity of urothelial cancer, and new standards of care have been established for the treatment of patients with metastatic disease. Iterative cycles of bench-to-bedside and bedside-to-bench research will further build on the findings of these important papers, and the pace of research progress in this historically understudied malignancy will continue to accelerate.

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UTUC IN 2017

Emerging evidence on treating upper tract urothelial cancer

Pietro Grande and Morgan Rouprêt

Major advances in the management of all stages of upper tract urothelial carcinoma have been made in 2017. Radical nephroureterectomy can be valuable in patients with metastatic disease and adjuvant platinum-based chemotherapy can improve outcomes in those with advanced disease. Kidney-sparing surgery with early follow-up ureterorenoscopy has shown benefit in patients with low-grade tumours. Avoiding unnecessary ureterorenoscopy might decrease intravesical tumour recurrence.

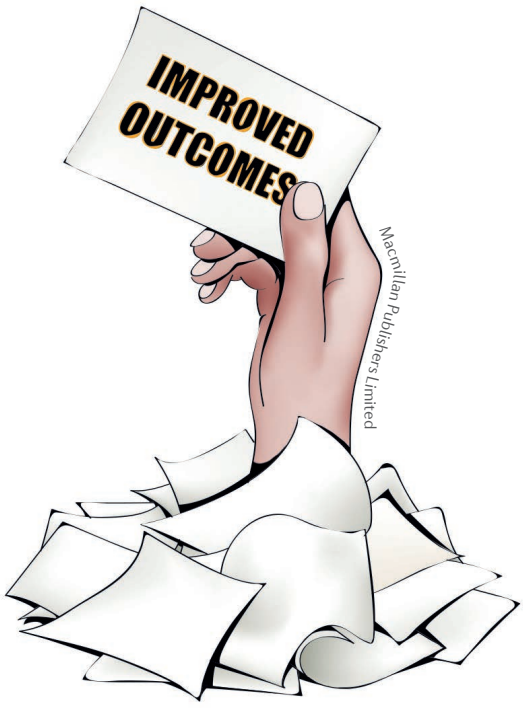
In comparison with urothelial bladder cancer, upper tract urothelial carcinoma (UTUC) is relatively uncommon. However, ~60% of UTUCs are invasive at diagnosis, compared with only 15–25% of bladder tumours[1]. UTUC accounts for 5% of all urothelial carcinomas and <10% of renal tumours and has its peak incidence in individuals aged 70–80 years. Radical nephroureterectomy (RNU) with bladder cuff excision is the standard of care in patients with high-risk disease[1], but conservative management has been proposed for small localized tumours. The use of adjuvant platinum-based chemotherapy for high-risk disease, although effective, is still a matter of debate. During the past 12 months, several papers on this topic report new evidence and advances in available treatment options.

Seisen and colleagues[2] retrospectively analysed the benefit of RNU in addition to platinum-based chemotherapy in patients with metastatic UTUC (mUTUC) over a median follow-up period of 25 months. Patients receiving chemotherapy only were compared with patients receiving RNU and chemotherapy. Data analysis was adjusted with inverse probability treatment weighting (IPTW) to avoid a potential selection bias among the two groups. IPTW Kaplan–Meier curves showed that 3-year overall survival was 16.2% in those receiving RNU and chemotherapy compared with 6.4% in those receiving chemotherapy only. Patients with mUTUC who received RNU and adjuvant chemotherapy were 30% less likely to die within 12 months following surgery; however, subset analyses showed that this benefit was reduced in patients with visceral metastasis compared with those who had nodal metastasis only. This finding confirms the survival benefit conferred by debulking surgery in UTUC, which had already been demonstrated for kidney cancer. Poor general patient health status and impaired renal function can restrict the possibility of kidney removal, and the consequent decrease in filtration rate can limit a subsequent use of chemotherapy. Hence, as Seisen *et al.*[2] suggest, accurate selection of patients for RNU is needed to achieve satisfactory results. In addition, the optimal sequence of RNU and chemotherapy (whether adjuvant or neoadjuvant) remains unclear.

Further clarification of the role of adjuvant chemotherapy in patients with locally advanced UTUC and/or positive regional lymph nodes was provided by another paper from Seisen and colleagues[3]. The team conducted a large IPTW-adjusted analysis of data from the US National Cancer Database of almost 3,500 patients with pT3/T4 and/or pN+ UTUC. The patients had received RNU followed by either observation ($n=2,500$) or adjuvant chemotherapy ($n=750$). The median follow-up period was 49.5 months. IPTW-adjusted Kaplan–Meier curves showed significantly longer median overall survival for patients who received adjuvant chemotherapy compared with those who underwent observation only (47.41 months versus 35.78 months; $P<0.001$). After a median follow-up duration of 4 years, patients in the adjuvant chemotherapy group were nearly 25% less likely to die, which translated into a mean extra survival benefit of 12 months for patients receiving adjuvant chemotherapy compared with those who underwent observation. Multivariate analyses taking into account possible confounding factors revealed that age, gender, Charlson Comorbidity Index, pathological stage, and surgical margin status had no significant effect on the outcome. This study is the first to establish a benefit of adjuvant chemotherapy, but the level of evidence for this finding remains low as no data were from randomized controlled trials.

The European Association of Urology (EAU) non-muscle invasive bladder cancer panel published a systematic review that compared kidney-sparing surgery (KSS) and RNU for UTUC[4]. The authors reported level 3 evidence that elective ureterorenoscopic KSS was not inferior to RNU in patients with low-grade disease with regard to 5-year and 10-year cancer-specific survival. In addition, the review reported similar rates of local-recurrence-free survival, bladder-recurrence-free survival, and metastasis-recurrence-free survival for both approaches. Thus, KSS emerges as the first-choice treatment for patients with low-risk disease who have unifocal, low-grade, noninvasive tumours. This review is based on data from retrospective comparative studies; hence, although the authors made great efforts to avoid any risk of bias, the report cannot offer definitive advice on the efficacy of segmental ureterectomy in comparison with RNU. Overall, the data suggest a potential for segmental ureterectomy to replace RNU in select patients with high-risk UTUC.

Villa and colleagues[5] proposed an interesting approach for the follow-up monitoring



Key advances

- Radical nephroureterectomy (RNU) is a valuable option for patients with metastatic upper tract urothelial carcinoma (UTUC) when combined with chemotherapy and can improve 12-month overall and cancer-specific survival[2]
- Adjuvant chemotherapy after RNU results in improved overall survival in patients with pT3/T4 and pN+ disease[3]
- Kidney-sparing surgery should be performed only in patients with low-grade disease, as current evidence suggests that segmental ureterectomy could be of value in highly select cases[4]
- An early secondary procedure in endoscopically treated patients with UTUC revealed a tumour-recurrence rate of 51.2%; secondary findings from ureterorenoscopy affected the outcomes of future endoscopic evaluations and the probability of undergoing subsequent RNU[5]
- Ureterorenoscopy resulted in increased intravesical recurrence rates[6]; thus, unnecessary ureterorenoscopy should be avoided to reduce the risk of intravesical recurrence and compromising patient outcomes
- Confocal laser endomicroscopy for ureterorenoscopy and percutaneous management of UTUC could aid in treatment decision-making and improve patient stratification and follow-up monitoring[7]

of patients treated with KSS for UTUC. In bladder cancer, early repeated transurethral resection of the bladder tumour (TURBT) is proposed at 2–6 weeks after the primary intervention. The authors analysed whether a similar approach could be beneficial in endoscopically treated patients with UTUC. The researchers performed second-look flexible ureterorenoscopy (URS) in 68 patients within 6–8 weeks after surgery with the aim of evaluating the oncological outcome of an early repeated URS and assessed the effect of this procedure on the need for further surgery. In 50% of patients who had cancer recurrence at second-look URS, the recurrence was located in the same area as the primary tumour. This finding suggests that incomplete eradication of UTUC during KSS can be overcome using early second-look URS to help detect early recurrence and to better characterize tumour aggressiveness. Thus, Villa *et al.*[5] concluded that the findings from early second-look URS affected subsequent endoscopic evaluations and the probability of undergoing subsequent RNU. Furthermore, for those patients who had an unsatisfactory pathological examination (owing to insufficient material for detailed analysis), the finding of second-look URS could be considered a surrogate marker for tumour grade. These results need to be corroborated in a study with a larger number of patients — possibly in a randomized controlled trial setting — but Villa *et al*.[5] have certainly demonstrated the need for better follow-up strategies after KSS.

Marchioni and colleagues[6] conducted a systematic literature review and meta-analysis on the role of preoperative diagnostic URS. Their findings support the hypothesis that this procedure is associated with an increased risk of intravesical recurrence. In the studies they assessed, intravesical recurrence rates were 39.2–60.7% and 16.7–46% in patients with and without previous URS, respectively. The pooled analyses showed a statistically significant association between performing URS before RNU and intravesical recurrence (HR 1.56, 95% CI 1.33–1.88; $P < 0.001$). The team observed no heterogeneity when the studies were compared with I^2 statistics of 2% ($P = 0.40$). Despite increased intravesical recurrence, preoperative URS did not affect recurrence, progression, or distant metastasis. Accordingly, we should select patients more carefully when proposing diagnostic URS. Patients who cannot benefit from organ-sparing surgery could be at risk of future intravesical recurrence, worsening their quality of life. However, Marchioni *et al.*[6] did not investigate possible benefits of flexible over semi-rigid URS.

A new and promising confocal laser endomicroscopy (CLE) tool (Cellvizio system, Mauna Kea Technologies, Paris, France) was tested by Breda and colleagues[7]. The researchers reported that CLE was promising for the endourological management of UTUC and helped provide highly accurate intraoperative information on tumour grade. In addition, the procedure required minimal training for an experienced endourologist. Inter-rater agreement between CLE and histopathological findings was substantial (Cohen's κ coefficient = 0.64). This technology could aid the treatment decision-making process and improve stratification of patients who could benefit from conservative treatment of UTUC. In addition, CLE could be employed during the follow-up monitoring of patients who had previously been managed conservatively, as well as those with carcinoma *in situ*. Large validation studies are needed, but CLE could be used more extensively for KSS, providing better diagnostics than conventional URS and improving follow-up monitoring.

Overall, in 2017, RNU proved to be an effective treatment in patients with mUTUC, and adjuvant chemotherapy after RNU was shown to increase overall survival in those with locally advanced disease. KSS resulted in similar outcomes as RNU in patients with low-grade tumours. Early second-look URS at 6–8 weeks after surgery is advised for patients undergoing KSS. Preoperative URS could lead to increased rates of intravesical recurrence and should, therefore, be reserved for select candidates for KSS. The CLE Cellvizio system shows promise in improving diagnosis and follow-up monitoring of UTUC.

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The authors declare no competing interests.

SURGERY IN 2017

Moving towards successful penile transplantation programmes

Jeffrey D. Campbell and Arthur L. Burnett

Implementing a successful penile transplantation programme requires a multidisciplinary approach to overcome social, institutional, and patient-related obstacles. The literature in 2017 presents controversial ethical solutions, novel research models to evaluate immunosuppression, and long-term patient reports that help advance the field towards developing successful penile transplantation programmes.

Patients with severe penile deformities often suffer debilitating physical symptoms and psychosocial distress, which ultimately disrupts their quality of life and interpersonal relationships. Although phalloplasty has been the traditional reconstructive approach for men with penile deficiencies, the procedure is limited by long-term complications, variable functional outcomes, cosmetic discrepancies, and poor availability of donor sites owing to traumatic limb amputation[1]. In the era of vascularized composite allotransplantation (VCA), penile transplant from a deceased donor has been proposed as a potential alternative.

The literature in 2017 presents several important issues surrounding the implementation of a successful penile transplantation programme. 'Success' in the arena of penile transplant goes far beyond proposing a programme or assembling a surgical team. Particular considerations include social, psychological, and ethical concerns; patient selection issues; technical surgical requirements; postoperative complication risks; immunosuppression prerequisites; and long-term functional and quality-of-life objectives[2]. A hierarchy of steps needs to be accomplished for penile transplantation programme success (FIG. 1).

The ethical considerations of penile VCA reflect the persistent controversy surrounding organ transplantation for improvement of recipient quality of life. Currently, no guidelines are available to mitigate the ethical concerns associated with this novel form of VCA. In an article published in 2017, a multidisciplinary team from New York reviewed institutional, social, and patient factors of clinical and research relevance and issued recommendations regarding selection of organ donors and recipients for penile transplantation, informed consent, financing, psychosocial support, and institutional review board involvement[3]. The families of potential donors should receive counselling and information regarding confirmation of the donor's death and the process of harvesting the donor tissue, and supporting institutions should accept the costs of this complex surgical procedure. Transplant candidates are directed to undergo a thorough psychosocial assessment and screening to set expectations and confirm social support mechanisms, mental health stability, economic resources, tolerance for publicity and stigma, and residence near the transplant team before the procedure is considered. Psychosocial assessment and social support are critical to the transplantation process and will be required for long-term care. Finally, the authors of this study stress the need for permission to publish all results, regardless of whether they are positive or negative, for public disclosure and progress of the field.

> "Psychosocial assessment and social support are critical to the transplantation process"

Under the United Network for Organ Sharing (UNOS) and the Organ Procurement and Transplantation Network (OPTN), VCA transplantation requires specific donor authorization, so public awareness and acceptance are essential. Rodrigue *et al.*[4] explored public attitudes towards VCA via an online survey that was accessible to US workers through Amazon Mechanical Turk. This nonvalidated questionnaire assessed the public's willingness to donate organs. Participants were educated on the standard practice for organ donation and the specific requirements for VCA transplantation. Remarkably, penis donation for transplant received a mere 50% willingness score and, along with face transplant, it had the highest "not at all willing" score, whereas solid organ transplant had a nearly 90% willingness score. The most common reason for the opposition towards penis donation was psychological discomfort[4]. This article highlights the negative public attitudes towards VCA donation, which remains a huge challenge for the penile transplant community and requires development of a coordinated approach for public education regarding VCA organ transplantation.

Penile transplantation requires surgical expertise, anatomical planning, and multidisciplinary team dedication to patient selection and surgical care coordination. In

Key advances

- Preliminary recommendations for ethical guidelines have been proposed for penile transplantation, with an emphasis on psychosocial support, institutional resources, patient selection, public awareness, and academic transparency[3]
- The general population seems uninformed and has a relatively low willingness for vascularized composite allotransplantation (VCA) organ donation; thus, improved public education regarding penile transplantation is necessary[4]
- The first successful penile transplant in the USA was performed at Massachusetts General Hospital in 2016 and demonstrated satisfactory 6-month functional outcomes[5]
- A novel *ex vivo* model of penile transplant rejection successfully demonstrated that tacrolimus and cyclosporine A prevent tissue rejection; however, tacrolimus demonstrated superior smooth muscle relaxation[6]
- After 2 years' follow-up duration, satisfactory urinary and sexual function results were reported from the first human penile allotransplant in South Africa[7]

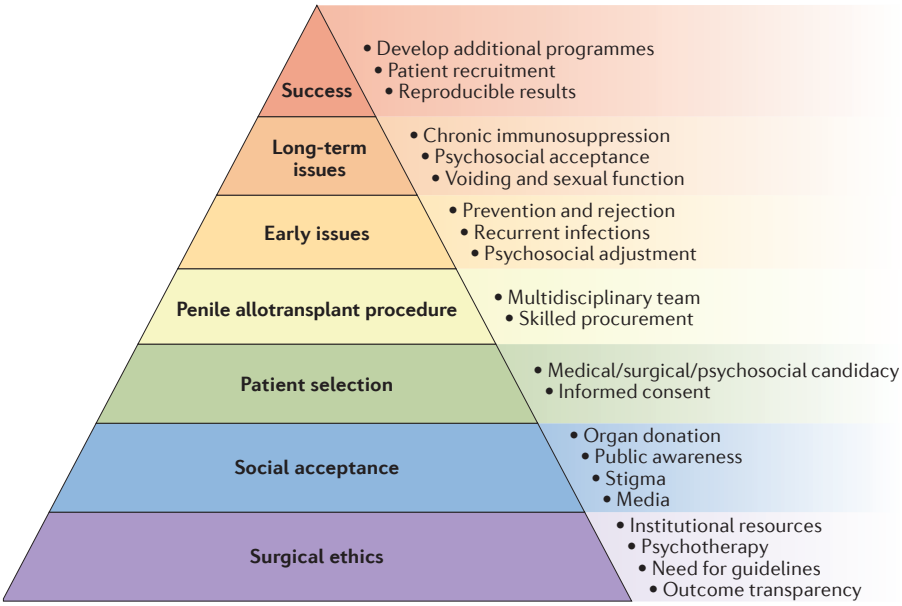

Figure 1 | **A hierarchy of steps need to be accomplished for success of a penile transplantation programme.** First, surgical ethics must be taken into consideration and social acceptance is required to ensure that potential donors and their families are willing to take part in the programme. Potential candidates for transplantation need to be recruited and then once all these features are in place the transplant procedure itself can be optimized. Both short-term and long-term adverse effects of a penile transplant for the patient must be considered and minimized before the programme can be considered a success.

2017, Cetrulo Jr. *et al.*[5] presented 6-month follow-up data for the first US human penile allotransplant, which was successfully performed in Boston at Massachusetts General Hospital in May 2016. Penile transplantation was performed for a patient who had previously undergone penectomy for penile cancer. After some initial postoperative issues with acute rejection and a small area of graft necrosis that required debridement, he was successfully discharged from the hospital 3 weeks after receiving the VCA. At the 6-month follow-up point, the patient had recovered penile sensation, partial erectile function, and adequate voiding function[5]. This report exemplifies the function of a superb multidisciplinary care team both in the preoperative and postoperative setting.

> “2017 has focused on minimizing the risk of organ rejection after penile transplantation”

Preventing long-term sequelae of organ rejection through chronic immunosuppression is a constant concern for patient care teams, and research activity in 2017 has focused on minimizing the risk of organ rejection after penile transplantation. Sopko *et al.*[6] prepared a mixed lymphocyte reaction (MLR) using cultured human cavernous tissue to create an *ex vivo* model of transplantation and rejection. They combined cavernous tissue with peripheral blood mononuclear cells from either the same patient or a donor to simulate autotransplantation and allotransplantation, respectively. Graft rejection impaired cavernous tissue physiology and was associated with cellular infiltration and apoptosis. Cultures were treated with selected immunosuppressants, and both tacrolimus (FK506) and cyclosporine A effectively prevented rejection. However, only FK506 preserved the integrity of the cultured erectile tissue, indicating that it is a more appropriate choice of immunosuppressant therapy for men who have received a penile transplant[6]. Further work using this model is warranted to define the immunosuppressant regimen for penile transplantation that will optimize immunological and functional outcomes.

Van der Merwe *et al.*[7] in Cape Town, South Africa, published their 2-year follow-up data from the first recorded human penile allotransplant, which was performed in December 2014 for a 21-year-old man suffering from a penile deformity secondary to ritual circumcision. Complications after the transplant included penile dorsal artery thrombosis and a urethrocutaneous fistula, which was primarily repaired. At 24 months postoperatively, no episodes of rejection had been observed and patient immunosuppression compliance was confirmed by serum testing. The patient reported return of erectile function at 3 weeks postoperatively and satisfactory penetrative intercourse after 5 weeks. The patient described normal skin sensation, ejaculation, orgasm, and achievement of a pregnancy with his partner. He had a significantly improved self-reported quality of life and was satisfied with the cosmetic outcome. The long-term success of this human penile transplantation is encouraging and supports ongoing penile transplantation endeavours.

Overall, the work done in 2017 has helped lay the groundwork towards advancing penile transplantation. Next steps will include establishing concrete ethical guidelines, optimizing immunosuppression, and defining coordinated multidisciplinary teams striving for the best psychological and functional outcomes. We have achieved a milestone in transplant history that could substantially improve the quality of life of patients with severe penile deficiencies.

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Both authors researched data for the article, made a substantial contribution to discussion of content, wrote the manuscript, and reviewed and edited the manuscript before submission.

Competing interests statement
The authors declare no competing interests.

KIDNEY CANCER IN 2017

Challenging and refining treatment paradigms

Mark W. Ball and Ramaprasad Srinivasan

Treatment paradigms for advanced renal cell carcinoma (RCC) continue to be challenged and refined. Recent studies in metastatic RCC have demonstrated the efficacy of first-line cabozantinib and the safety and efficacy of dual checkpoint blockade; in the adjuvant setting, pazopanib failed to improve progression-free survival in high-risk localized RCC compared with placebo.

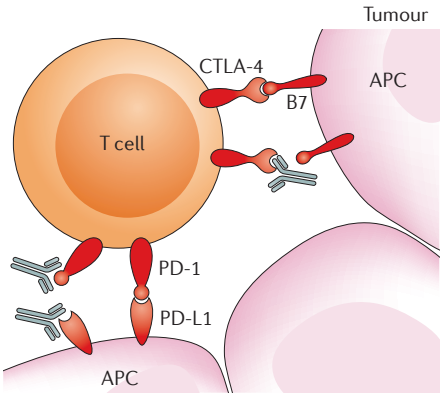
Renal cell carcinoma (RCC) continues to be a considerable global burden, leading to >140,000 deaths and affecting >300,000 individuals worldwide each year[1]. Currently, 11 systemic therapies have received FDA approval for treating metastatic RCC (mRCC). No new therapies received FDA approval in 2017, but three important studies sought to challenge existing treatment paradigms by evaluating the role of cabozantinib in the first-line setting for mRCC[2], the safety and efficacy of dual checkpoint inhibition with nivolumab and ipilimumab for mRCC[3], and the role of adjuvant pazopanib for high-risk, nonmetastatic RCC[4].

> three important studies sought to challenge existing treatment paradigms

First-line treatment options for mRCC currently include sunitinib, pazopanib, bevacizumab plus interferon-α, high-dose IL-2, and, for patients with poor-risk disease, temsirolimus[5]. Cabozantinib is an oral tyrosine kinase inhibitor that targets the vascular endothelial growth factor (VEGF), proto-oncogene c-Met (MET), and AXL oncogene (AXL) pathways. It initially gained approval for treating patients who progressed on VEGF receptor (VEGFR) inhibitors after a phase III trial demonstrated superior progression-free survival (PFS) of patients receiving cabozantinib (60 mg daily) compared with everolimus (10 mg daily; median PFS 7.4 versus 3.8 months, HR 0.51, $P < 0.0001$)[6]. CABOSUN (NCT01835158) was a randomized phase II trial that compared cabozantinib (60 mg daily) with sunitinib (50 mg daily, 4 weeks on, 2 weeks off) in 157 patients with previously untreated intermediate-risk or poor-risk mRCC[2]. Median PFS was longer with cabozantinib than with sunitinib (8.2 versus 5.6 months, HR 0.66; 95% CI 0.46–0.95; one-sided $P = 0.012$), as was the overall response rate (ORR, 46%; 95% CI 34–57% versus 18%; 95% CI 10–28). Furthermore, the incidence of grade 3–4 adverse events was similar between arms (67% for cabozantinib and 68% for sunitinib). Based on these data, in December 2017, the FDA broadened the indications for cabozantinib to include previously untreated patients. These results are promising, but demonstration of superior efficacy in a phase III trial against a current standard is required to definitively establish the role of cabozantinib in the first-line setting.

A resurgence of interest has occurred in evaluating immunotherapy in mRCC with the recent introduction of immune checkpoint inhibitors that block programmed cell death protein 1 (PD1) and cytotoxic T lymphocyte-associated protein 4 (CTLA4). PD1 is a cell-surface protein in the B7–CD28 family that is expressed on both activated and exhausted T cells. Nivolumab is a monoclonal antibody directed against PD1, which gained FDA approval to treat mRCC in the second-line setting after it was shown to improve overall survival compared with everolimus[7]. CTLA4 is expressed by T cells, preventing them from receiving the co-stimulatory signal mediated by B7–CD28 interaction; blockade of CTLA4 leads to T cell activation. Ipilimumab is a monocolonal antibody against CTLA4. Dual PD1 and CTLA4 inhibition has synergistic activity in multiple solid malignancies[8].

CheckMate 016 (NCT01472081) was a multi-arm phase I, dose-escalation study that evaluated the combination of nivolumab and ipilimumab in both treatment-naive and previously treated patients with mRCC[3]. Results from three treatment arms were recently reported: nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (arm N3I1), nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (arm N1I3), or nivolumab 3 mg/kg plus ipilimumab 3 mg/kg (arm N3I3) every 3 weeks for four doses followed by nivolumab monotherapy 3 mg/kg every 2 weeks until progression. Notably, only six patients were treated in the N3I3 arm owing to high toxicity (five of six patients experienced a grade 3–4 adverse event). The N3I1 and N1I3 arms included 47 patients each and the treatment regimens were better tolerated than in N3I3. Grade 3–4 adverse events were reported in 38.3% and 61.7% of patients in the N3I1 and N1I3 arms, respectively. The ORR was 40.4% in both the N3I1 and N1I3 arms. Moreover, responses were durable: ongoing responses were seen in 42.1% and 36.8% of responders in the N3I1 and N1I3 arms, respectively, (median follow-up duration of 22.3 months). In the N3I1 arm, five patients (10.6%) achieved a complete response and 14 (29.8%) had a partial response. In the N1I3 arm, 19 (40.4%) patients had a partial response, but no complete responses were reported. At 24 months, overall survival was 67% and 70% in the N3I1 and N1I3 arms, respectively.



Key advances

- Cabozantinib was shown to be superior to sunitinib with similar adverse event rates among patients with intermediate-risk or poor-risk metastatic renal cell carcinoma (RCC) in the phase II trial CABOSUN[2]
- Dual checkpoint inhibition with nivolumab and ipilimumab was shown to be efficacious with manageable toxicity in patients with metastatic RCC in the phase I study CheckMate 016 (REF. 3)
- Adjuvant pazopanib after nephrectomy for high-risk localized RCC did not improve disease-free survival compared with placebo in the phase III study PROTECT[4]

Initial experiences with combination checkpoint blockade seemed to indicate unacceptable toxicity, but CheckMate 016 demonstrated that the particular dosing regimen is a major factor in determining toxicity. However, the ~10% complete response rate seen in the N3I1 arm is very encouraging. Currently, the only FDA-approved therapy that is consistently associated with complete responses is high-dose IL-2, but at the cost of high toxicity. Whether the complete responses seen in the N3I1 arm can lead to long-term remissions is unclear, but the data from this study provided the basis for CheckMate 214, a phase III trial evaluating this regimen versus sunitinib in the first-line setting. Preliminary data from this study indicated that patients with intermediate-risk or poor-risk disease who received the N3I1 regimen had improved PFS compared with those who received sunitinib, whereas patients with favourable-risk disease patients had better PFS with sunitinib.

The role of adjuvant therapy after nephrectomy for high-risk localized RCC remains unclear. Two recent trials — adjuvant sorafenib and sunitinib for unfavourable renal carcinoma (ASSURE)[9], and sunitinib as adjuvant treatment for patients at high risk of recurrence of RCC following nephrectomy (S-TRAC)[10] — demonstrated conflicting results. Pazopanib as adjuvant therapy in localized or locally advanced RCC after nephrectomy (PROTECT, NCT01235962) was a phase III trial that randomly assigned 1,538 patients with nonmetastatic, high-grade pT2 or >pT3 including N1 clear cell or clear-cell-predominant RCC to pazopanib or placebo for 1 year with a primary end point of disease-free survival (DFS)[4]. The trial was initially designed to use a starting dose of 800 mg of pazopanib, but it was lowered to 600 mg after 403 patients already enrolled in the treatment arm experienced toxic effects. In the intention-to-treat analysis of the cohort that received 600 mg ($n = 1,135$), no DFS benefit was reported for pazopanib versus placebo either in the primary analysis (HR 0.86; 95% CI 0.70–1.06; $P = 0.165$) or in the follow-up analysis 12 months later (HR 0.94, 95% CI 0.77–1.14). In the secondary analysis of DFS in patients who received 800 mg ($n = 403$), DFS was improved in the pazopanib arm (HR 0.69, 95% CI 0.51–0.94). Grade 3–4 adverse events were seen in 60% of the pazopanib 600 mg cohort, 66% of the 800 mg cohort, and 21% of the placebo cohort. Treatment-related discontinuations occurred in 35% and 39% of patients in the 600 mg and 800 mg arms, respectively. Elevated transaminases were the most common adverse events resulting in treatment discontinuation.

Considerable differences exist in the design of the three completed studies of adjuvant therapy in RCC, but the results of PROTECT are consistent with those reported in ASSURE. ASSURE was a phase III trial of 1,943 patients with high-grade T1b or higher-stage nonmetastatic RCC randomized to receive sunitinib, sorafenib, or placebo for 54 weeks. No improvement in PFS was seen in the adjuvant arms compared with placebo (median PFS 5.8 years, HR 1.02, $P = 0.8$ for sunitinib versus 6.1 years, HR 0.97, $P = 0.7$ for sorafenib versus 6.6 years for placebo). Treatment-related discontinuation was 44% and 45% in the sunitinib and sorafenib arms, respectively. By contrast, S-TRAC was a phase III trial that randomized 615 patients with T3 or higher-stage RCC to sunitinib versus placebo for 1 year, and the results demonstrated improved DFS for sunitinib compared with placebo (6.8 versus 5.8 years, $P = 0.03$)[10]. Treatment-related discontinuation was only 28.1% in S-TRAC, which was lower than in ASSURE and PROTECT.

Reconciling the results of these three adjuvant trials is difficult given the difference in trial design. S-TRAC is the only trial in which adjuvant therapy provided a benefit; thus, evaluating how it differed from the other two trials might be instructive. Patients included in S-TRAC had the highest disease stages of all three trials, as all patients had T3–T4 disease, whereas the proportion of patients with T3–T4 disease in PROTECT and ASSURE was 85% and 65%, respectively. Furthermore, treatment-related drug discontinuation was lowest in S-TRAC; hence, patients in this trial received the highest amounts of drugs. However, whether patients ultimately derive benefit from extended DFS is unclear. Given that none of these agents improve overall survival in the metastatic setting, whether administering these agents in the adjuvant setting rather than at relapse will alter the natural history of the disease is unknown. Thus, this treatment regimen does expose patients to a high burden of toxicity. At this time, the utility of adjuvant therapy in RCC remains a topic of considerable debate. However, the FDA approved the adjuvant use of sunitinib in adult patients at high risk of recurrent renal cell carcinoma following nephrectomy.

Several studies reported this year have sought to refine RCC treatment regimens, but questions remain. The optimal first-line therapy for clear cell RCC, the sequencing of later-line therapies, treatment of non-clear-cell RCC, the role of adjuvant therapy, and the selection of appropriate candidates for immunotherapy are active areas of investigation. Future studies to address these knowledge gaps, coupled with a search for biomarkers to guide therapy at all stages will continue to improve treatment strategies in RCC.

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Competing interests statement
The authors declare no competing interests

TESTICULAR CANCER IN 2017

Sequencing advances understanding

Matthew J. Murray and Clare Turnbull

Our biological understanding of TGCTs has been improved using sequencing, and molecular profiles associated with the genomic evolution and development of cisplatin resistance have been identified. The genomics of variants underpinning TGCT predisposition is being delineated. Studies of circulating microRNAs have demonstrated their potential for noninvasive diagnosis and disease monitoring.

The incidence of testicular germ cell tumour (TGCT) is increasing and is a leading cause of death in young men. The introduction of cisplatin therapy 40 years ago resulted in improved survival for metastatic TGCT. Stratification of metastatic disease into good-risk, intermediate-risk, and poor-risk groups via the International Germ Cell Consensus Classification has, for the past 20 years, facilitated standardization of treatment and direct comparison of clinical trial results. However, escalation of therapy within the intermediate-risk and/or poor-risk groups has not generally resulted in clear survival advantages. Furthermore, attempts to identify additional clinical and/or tumour markers to predict platinum resistance have largely failed.

The end of 2016 saw publication of long-awaited landmark first insights into platinum resistance, afforded by large-scale agnostic next-generation sequencing (NGS). An analysis of 180 cisplatin-resistant and cisplatin-sensitive germ cell tumours, in which discovery whole-exome sequencing (WES) in 19 samples and targeted exon-capture-based sequencing of 300 genes in 161 samples was undertaken, was performed[1]. This study confirmed the low mean rate of small mutations (0.9/Mb) with the only frequent recurrent finding in TGCTs being gain of chromosome 12p (REF. 2). Distinguishing features of cisplatin-resistant compared with cisplatin-sensitive tumours were reported: elevated median number of small mutations and enrichment for *TP53* mutation or amplifications in the *TP53* regulator gene *MDM2*. Furthermore, WES was performed on 59 tumours from 51 patients with either platinum-sensitive or platinum-resistant tumours, including serial primary and metastatic sites where available[3]. Notably, all TGCTs had wild-type *TP53* with expression maintained in both primary and metastatic tumours. This report is the first to show a strikingly high frequency of reciprocal loss-of-heterozygosity copy-number-neutral events in TGCTs, which is much higher than other cancers, and which the researchers replicated in an independent series[2]. The hallmark trio of genomic characteristics in TGCTs were defined as wild-type *TP53*, chromosome arm 12p gain, and additional reciprocal copy number changes, and similarity to adaptive mutations acquired by human embryonic stem cells was highlighted[3]. The investigators hypothesized that the platinum sensitivity of TGCTs could be associated with high mitochondrial priming and demonstrated an increase in BIM BH3-induced mitochondrial depolarization in TGCTs[3]. Disappointingly, but probably unsurprisingly, these analyses did not converge upon a single neat biomarker of platinum resistance, nor a ready therapeutic target. However, together they offer biological insights into platinum sensitivity; additional depth may be gleaned from broader 'multi-omic' profiling.

Improved detection and disease monitoring is another clinical priority. Conventional tumour markers (AFP, HCG, and LDH) have been used effectively for early risk stratification and detection of relapse in nonseminomatous GCTs but have limited sensitivity and specificity for patients with seminoma. Circulating microRNAs have had great promise as universal markers for diagnosis and disease monitoring for malignant GCTs since their first description in 2011 (FIG. 1) (REFS 4,5).

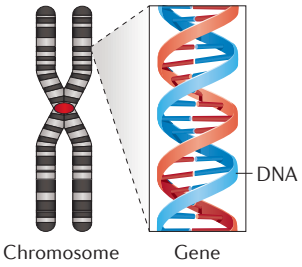
Using the highly sensitive multiplexed preamplification qRT–PCR technique[4], serum levels of a panel of four microRNAs (miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p) were analysed at primary diagnosis of 166 patients with TGCT and 118 men without[6]. Considerably higher serum expression levels for each of the microRNAs was seen in patients with malignant TGCT than in those without[6]. Notably, levels of miR-371a-3p fell to normal after successful completion of treatment, with persistently elevated values in patients who experienced treatment failure and relapse. Using the density estimation model, the sensitivity, specificity, and AUC for miR-371a-3p in the whole cohort were 89%, 93%, and 0.95, respectively, outperforming the conventional serum markers (which have a combined sensitivity of 50%)[6]. Analyses of three microRNAs (miR-371a-3p, miR-373-3p, and miR-367-3p) in 250 primary TGCT samples (seminomas and nonseminomatous GCTs) and 164 nonmalignant samples[7] similarly showed the sensitivity, specificity, and AUC of these microRNAs combined to be 90%, 91%, and 0.96, respectively; for miR-371a-3p alone these parameters were 90%, 86%, and 0.95, respectively[7]. These studies indicate that miR-371a-3p might ultimately offer the most clinical utility, although the rationale for assessing the full panel of all four in prospective trials has been highlighted[5]. These two large studies confirm previous reports demonstrating improved sensitivity and specificity of microRNAs compared with conventional serum markers. Validation in prospective clinical trials is

Key advances

- Large-scale exome sequencing studies of testicular germ cell tumours (TGCTs) revealed distinctive patterns of reciprocal chromosome loss, indicating potential mechanisms underpinning platinum sensitivity[1,3]
- Large-scale germline exome sequencing of multicase TGCT families revealed a polygenic genomic architecture underlying this disease[8]
- Genome-wide association studies[9,10] have doubled the number of identified common genetic variants linked to TGCT susceptibility from 25 to 49
- Biomarker studies demonstrated the superiority of a panel of circulating microRNAs (including miR-371a-3p) over traditional serum markers for TGCT diagnosis and monitoring[6,7]

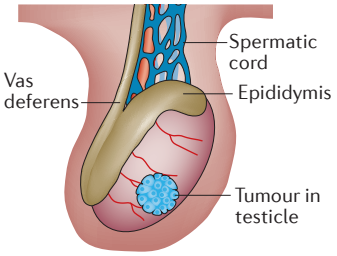
Genomic susceptibility

- Heritability is higher (49%) and familial relative risk greater (fourfold to eightfold) than for other cancers
- Susceptibility to testicular germ cell tumour (TGCT) is highly polygenic
- No major high-penetrance genes were found using exome sequencing of families
- Recent genome-wide association studies identified 49 common variants
- Implicated pathways include *KIT* signalling, transcriptional regulation of male germ cell development factors, and defective microtubule assembly



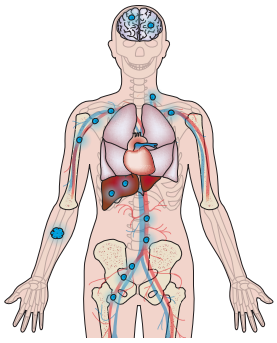
Somatic profiling of malignant tumours

- TGCTs have a low rate of somatic small mutations
- *KIT* and *KRAS* are the most frequent driver genes
- Wild-type *TP53* is typically retained
- A large number of copy number and structural variants are present in TGCTs
- i(12p) is the hallmark structural variant of TGCTs
- ***Features of seminoma:*** mutant *KIT*, hypertriploid
- ***Features of nonseminoma:*** wild-type *KIT*, hypotriploid
- Very distinctive patterns of copy-number-neutral reciprocal chromosome loss found in TGCTs



Understanding platinum sensitivity and resistance

- TGCTs are unusually and exquisitely platinum-sensitive (overall 5-year survival >95%)
- Mechanisms of platinum sensitivity are poorly understood
- The TP53 pathway (including MDM2) is likely to be important
- High mitochondrial priming could have a role in platinum sensitivity (as mitochondria have a high BCL2-related proapoptotic propensity)



Noninvasive microRNA monitoring for diagnosis and recurrence

- Current serum tumour markers (α-fetoprotein, human chorionic gonadotropin, and lactate dehydrogenase) have limited sensitivity and specificity, particularly for seminomas
- Quantitative real-time PCR of a panel of four circulating microRNAs is highly sensitive and specific for malignant TGCT diagnosis and/or disease-monitoring and relapse detection
- miR-371a-3p is the most individually predictive microRNA
- MicroRNAs could reduce the number of CT scans required in follow-up monitoring
- Prospective clinical trials are underway

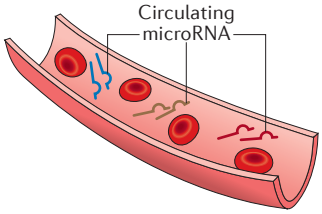


Figure 1 | **Testicular cancer in 2017 – advances in molecular understanding gained using large-scale sequencing.**

under way, heralding an opportunity for non-invasive monitoring of malignant TGCTs and reduced use of serial CT scans and consequent radiation exposure[5] (FIG. 1).

TGCT has a strong heritable basis. In December 2016, the largest series of germline WES to date, comprising 328 TGCTs from 153 families experiencing multiple instances of TGCT, was published[8]. No gene was found in which disruptive mutations were segregating in more than 3 of 153 families. The top hit was *DNAAF1*, but with mutations only segregating in two families. Implication of *DNAAF1* in TGCT tumorigenesis was supported by demonstration of second-hit mutations and loss of protein staining for *DNAAF1* in tumours, as well as in a *DNAAF1*$^{hu255h(+/-)}$ zebrafish model. Mutations in six related genes were found in 9 of 151 further multicase TGCT families[8]. Larger WES studies are required, integrating familial and simplex cases. However, these results suggest that the contribution of rare alleles to TGCT heritability is highly polygenic. Based on these findings, clinic-based genetic testing for assessing inherited TGCT risk is unlikely to be useful.

Most of the high heritability of TGCT is likely to be underpinned by common genetic variants. Results of genome-wide association studies (GWAS) in TGCT have been remarkably fruitful, identifying variants with some of the highest effect sizes reported across all cancer types. In the past year, two major GWAS analyses[9,10] have resulted in an increase in the total number of identified TGCT-associated common variants from 25 to 49. By genotyping samples from 7,319 men with TGCTs and 23,082 controls, 19 new TGCT-associated loci were identified[9]. Widespread disruption of developmental transcriptional regulators, defective microtubule assembly, and dysregulation of *KIT–MAPK* signalling were revealed via chromatin-interaction analysis as potential mechanisms key in early oncogenesis. A meta-analysis of five GWAS for TECAC[10] identified eight TGCT susceptibility loci, of which five were novel and three overlapped with those identified in the other GWAS[9,10] (FIG. 1).

The growing number of rare and common susceptibility alleles is complementing our expanding landscape of molecular determinants of TGCT development and treatment response. Translation of our advances in molecular understanding of TGCTs into clinical benefit for patients is the next challenge.

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Competing interests statement
The authors declare no competing interests.

PROSTATE CANCER IN 2017

Advances in imaging

Andreas G. Wibmer, Hebert Alberto Vargas and Hedvig Hricak

In the past year, the results of three studies in the field of prostate cancer imaging — the prostate MR imaging study (PROMIS), an analysis of the cost-effectiveness of various diagnostic strategies based on PROMIS data, and a retrospective analysis of a prostate-specific membrane antigen (PSMA)-directed PET radiopharmaceutical — have been published that could have lasting effects on clinical practice.

Over the past year, the scholarly literature on imaging of prostate cancer continued to expand rapidly: since October 2016, >1,400 articles on prostate cancer imaging have been listed on the PubMed database. Results from three papers in particular could have especially strong, sustained effects on clinical practice: the prostate MR imaging study (PROMIS)[1], an analysis of the cost-effectiveness of various diagnostic strategies based on PROMIS data[2], and a retrospective analysis of a prostate-specific membrane antigen (PSMA, also known as glutamate carboxypeptidase 2)-directed PET radiopharmaceutical[3].

> In the head-to-head comparison, prostate MRI was more sensitive than TRUS biopsy … and less specific

For diagnosis of suspected primary prostate cancer, the exact role of prostate MRI in relation to transrectal ultrasonography-guided (TRUS) biopsy has been debated for over a decade. PROMIS, published in February 2017, was a prospective multicentre investigation directly comparing the diagnostic precision of the two tests in a cohort of 576 men with suspected prostate cancer[1].

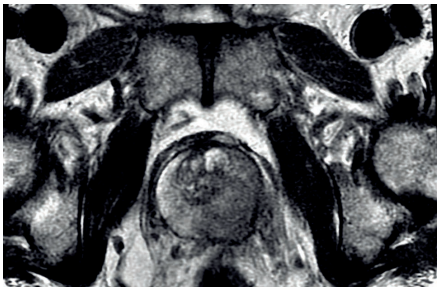
As part of the PROMIS protocol, biopsy-naive patients with elevated serum PSA, suspicious digital rectal examination, or a family history of prostate cancer underwent a standardized 1.5 T prostate MRI examination that comprised T1-weighted, T2-weighted, diffusion-weighted, and contrast-enhanced sequences. All study radiologists were specialists in urogenital imaging, underwent additional specific training before the start of the study, and were provided with the patients' clinical details. The likelihood of prostate cancer was rated on a 5-point Likert scale, ranging from 1 (highly unlikely) to 5 (highly likely), and an MRI score ≥3 was regarded as positive for the presence of prostate cancer (418/576, 73%). With patients, clinicians, and pathologists blinded to the MRI results, patients underwent combined prostate biopsy sampling, including sequential transperineal template prostate mapping (TPM, the reference standard) and 12-core TRUS biopsy. On histopathological examination, clinically significant prostate cancer was defined as the presence of any Gleason score ≥4 + 3 or any cancer core length ≥6 mm (230/576, 40%). In the head-to-head comparison, prostate MRI was more sensitive than TRUS biopsy (93% versus 48%, $P < 0.0001$) and less specific (41% versus 96%, $P < 0.0001$). These results translated to a significantly higher NPV (89% versus 74%, $P < 0.0001$) and significantly lower PPV (51% versus 90%, $P < 0.0001$) for MRI than for TRUS biopsy. TRUS biopsy missed 119 of 230 (52%) instances of clinically significant prostate cancer, whereas MRI missed 17 of 230 (7%) instances. Notably, MRI identified all cases with a Gleason score ≥4 + 3; the 17 cases missed on MRI were classified as clinically significant based on maximum cancer core length alone. The authors concluded that ~25% of patients with a clinical suspicion of prostate cancer and a negative MRI could safely forgo TRUS biopsy.

A companion publication evaluated the cost-effectiveness of various diagnostic strategies based on the PROMIS data[2]. This study evaluated 383 possible combinations of prostate MRI, systematic TRUS biopsy, MRI-targeted TRUS biopsy, and TPM biopsy. The analysis showed that, of all combinations with initial MRI and subsequent targeted TRUS biopsies of suspicious lesions, the most cost-effective strategies would detect 85–95% of all clinically significant cancers at a cost of £628–807 per patient. For TRUS-biopsy-first strategies, the most cost-effective, in which MRI and targeted biopsies would be performed only in patients with negative initial TRUS biopsy, would detect 91% of all clinically significant cancers at a cost of £709 per patient. The authors concluded that prostate MRI followed by targeted TRUS biopsy of suspicious lesions would be cost-effective for prostate cancer diagnosis.

PROMIS is the largest prospective trial providing level 1 evidence for the value of prostate MRI as a triage test in patients with clinical suspicion of prostate cancer. The most important finding from this study is that MRI seems to provide the high sensitivity and NPV needed for it to be a clinically useful screening tool. The results of PROMIS corroborate previous reports, as summarized in a 2017 meta-analysis of 48 studies (9,613 patients), among which the median NPV for clinically significant prostate cancer was 88%[4].

Reasonable arguments exist supporting and questioning the validity and applicability of the PROMIS results. The main criticism is that the high quality of MRI and expertise of radiologists involved in PROMIS would be difficult to achieve in general practice. In a real-world scenario, the sensitivity of prostate MRI would probably be lower than that reported in PROMIS. However, the prevalence of clinically significant prostate cancer in the PROMIS population (40%) was higher than would be expected in the general population (in fact, the sample size calculations for PROMIS were based on a prevalence of 15%). With lower disease prevalence in the tested population, the NPV of MRI would probably stay high, even with a slightly reduced sensitivity.

In the cost-effectiveness analysis, MRI-first strategies seem to be slightly more efficient but also more expensive than TRUS-first strategies. Caution is needed when translating these numbers into clinical practice. First, the calculations were exclusively based on the UK health-care system, which might be different from those of other regions. Second, cancer detection rates might be higher with computational MRI–ultrasonography-fusion biopsy, which is now routinely performed in many countries, than with cognitively targeted TRUS



Hedvig Hricak, Memorial Sloan Kettering Cancer Center

Key advances

- PROMIS provides level 1 evidence that prostate MRI has a higher sensitivity and lower specificity for the diagnosis of prostate cancer than transrectal ultrasonography-guided (TRUS) biopsy[1]
- The results of PROMIS substantiate the feasibility and safety of avoiding TRUS biopsy in patients with a negative MRI[1]
- Prostate MRI followed by targeted TRUS biopsy of suspicious lesions is a cost-effective strategy for prostate cancer diagnosis[2]
- PSMA-targeting PET tracers seem to provide unique sensitivity for the detection and localization of recurrent and metastatic prostate cancer[3]

biopsy[5], although some investigators have not found a significant difference between the two methods[6]. The efficacy of MRI-first strategies and their costs might depend, therefore, on the biopsy sampling technique used, which was not taken into account in the PROMIS cost-effectiveness analysis. In addition, changing the definition of clinically significant prostate cancer on histopathology will alter the diagnostic performance of prostate MRI, as shown in PROMIS and previous reports. However, whether biopsy findings alone can truly indicate clinical significance, which is ultimately defined by the occurrence of adverse clinical events (such as the development of symptomatic disease, need for therapy, or death from cancer) is questionable. For PROMIS, study participants consented to long-term follow-up monitoring for cancer outcomes and mortality. These data will hopefully enable assessment of the true value of prostate MRI, TPM biopsy, and TRUS biopsy, not only from a diagnostic perspective but also from a prognostic one.

> “the likelihood of a [PSMA-PET–CT] positive finding was almost exclusively determined by PSA level”

Regarding recurrent prostate cancer, a growing body of evidence suggests that patients with local tumour recurrence after radical treatment or oligometastatic disease might benefit from targeted therapies[7]. In this scenario, imaging is needed to localize disease sites. Up until a few years ago, the available conventional imaging methods, including CT, bone scintigraphy, or PET–CT with ^{18}F-labelled glucose or sodium fluoride, were of limited sensitivity, particularly in patients with early biochemical recurrence (such as PSA levels <1 ng/ml after prostatectomy)[8]. Some PET radiopharmaceuticals recently approved by the FDA, including ^{11}C-choline and ^{18}F-fluciclovine, are also of limited use in patients with such low PSA levels[9]. However, in 2011, PET probes targeting PSMA became available for clinical use. PSMA is highly overexpressed in prostate cancer. A retrospective analysis of a PSMA-directed PET radiopharmaceutical in a consecutive series of 1,007 patients with recurrent prostate cancer was published in May 2017[3]. Patients had initially been treated with surgery, radiotherapy, hormonal therapy, high-intensity focused ultrasound ablation, or a combination of these therapies with or without chemotherapy. All patients underwent a standardized PET–CT examination 1 hour after injection of ^{68}Ga-PSMA-11. The primary end point was positivity of the imaging study, defined as the presence of at least one radiotracer-avid lesion considered visually typical of prostate cancer, by the consensus of three nuclear medicine physicians and one radiologist. The reported positivity rate was 801 of 1,007 patients (80%), and multivariate analyses indicated that the likelihood of a positive finding was almost exclusively determined by PSA level: positivity rates ranged from 46% in patients with PSA ≤0.2 ng/ml to 57% for PSA levels ≤1 ng/ml, to >90% in patients with PSA >3 ng/ml.

To date, this study is the largest published clinical experience with any type of PSMA-directed imaging probe. Despite its retrospective design, the heterogeneous study cohort, and the lack of histopathological confirmation of positive findings, the results of this study are encouraging. The findings are in line with a 2016 meta-analysis (overall positivity rate of 81%, 50% in patients with PSA 0.2–0.49 ng/ml) in the recurrent prostate cancer setting (nine studies, 983 patients),[10] and compare favourably with positivity rates of ^{11}C-choline and ^{18}F-fluciclovine PET–CT reported in a recent prospective trial (for all patients: 37% and 34%, respectively; for patients with PSA <1 ng/ml: 14% and 21%, respectively)[9]. The lack of histopathological correlation is probably the most notable drawback of this study and the true sensitivity of PSMA-PET–CT is probably lower than reported, as the list of known PSMA-expressing benign and malignant tissues expands with the increasingly widespread use of PSMA-targeting tracers. Prospective trials with histopathological correlation are under way to test the true diagnostic precision of PSMA-targeting agents.

In summary, we believe PROMIS provides evidence for the safe avoidance of TRUS biopsy in patients with a negative prostate MRI, provided that technical adequacy and qualified radiologists are available. Furthermore, PSMA-targeting PET tracers are developing into the new standard for imaging of recurrent prostate cancer, but further controlled studies are needed to fully determine their strengths and limitations.

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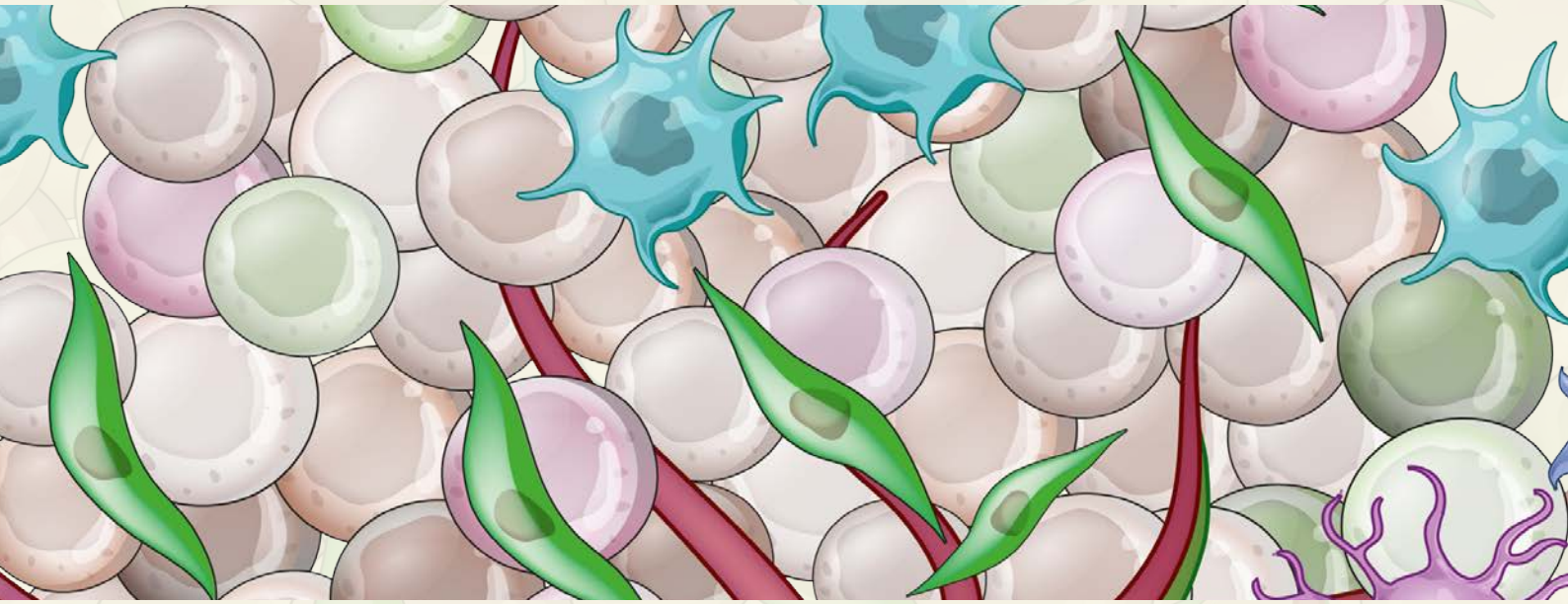

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