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Highlights

High hopes and high honours for cancer immunotherapy

Sophia Julia Häfner*

University of Copenhagen, BRIC Biotech Research & Innovation Centre, Anders Lund Group, Ole Maaløes Vej 5, 2200 Copenhagen, Denmark

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ABSTRACT

This issue of the *Biomedical Journal* honours the laureates of the 2018 Nobel Prize in Physiology and Medicine for their ground-breaking contributions to cancer immunotherapy and unveils the identification of essential intermediates between microtubule-targeting agents and apoptosis. Subsequently, we learn about the hypoglycemic properties of natural phenolic acids, how cone-beam computed tomography assists dental implant surgery and which factors should be taken into account for salvage liver transplantation after recurrent hepatocellular carcinoma. Further readings discuss the negative impact of bismuth shields on computed tomography image quality, the predictive value of warning headaches for aneurysmal subarachnoid haemorrhage and the great long-term performance of zirconia implant abutments.

Spotlight on reviews

High hopes and high honours for cancer immunotherapy

If there is one synonym for hope in the field of cancer therapy in these recent years, then it is immunotherapy [1]. According to the American Society of Clinical Oncology (ASCO), the “advance of the year” in 2018 was the use of chimeric antigen receptor (CAR) T cell immunotherapy for leukaemia and lymphoma, an approach consisting in genetically engineered T cells, equipped with a receptor that both recognises a specific antigen on the tumour surface, and acts as a T-cell activator [2]. Moreover, the use of tumour-associated antigens for

therapeutic cancer vaccines has lately experienced a regain in interest [3].

In any case, one indisputable key strategy of cancer immunotherapies over the last decades have been immune checkpoint inhibitors.

Fittingly, the Nobel Prize of Physiology and Medicine 2018 was awarded to two eminent figures in the field, Tasuku Honjo and James Allison, who worked respectively on the programmed death molecule-1 (PD-1) and the cytotoxic T-lymphocyte antigen 4 (CTLA-4). Both molecules are implicated in the suppression of T cell activation [Fig. 1] and their inhibition is nowadays at the core of several cancer therapies.

Therefore, Pei-Wei Huang and John Wen-Cheng Chang, themselves possessing broad experience in the fields of

* Corresponding author. University of Copenhagen, BRIC Biotech Research & Innovation Centre, Anders Lund Group, Ole Maaløes Vej 5 2200 Copenhagen, Denmark.

E-mail address: sophia.hafner@bric.ku.dk.

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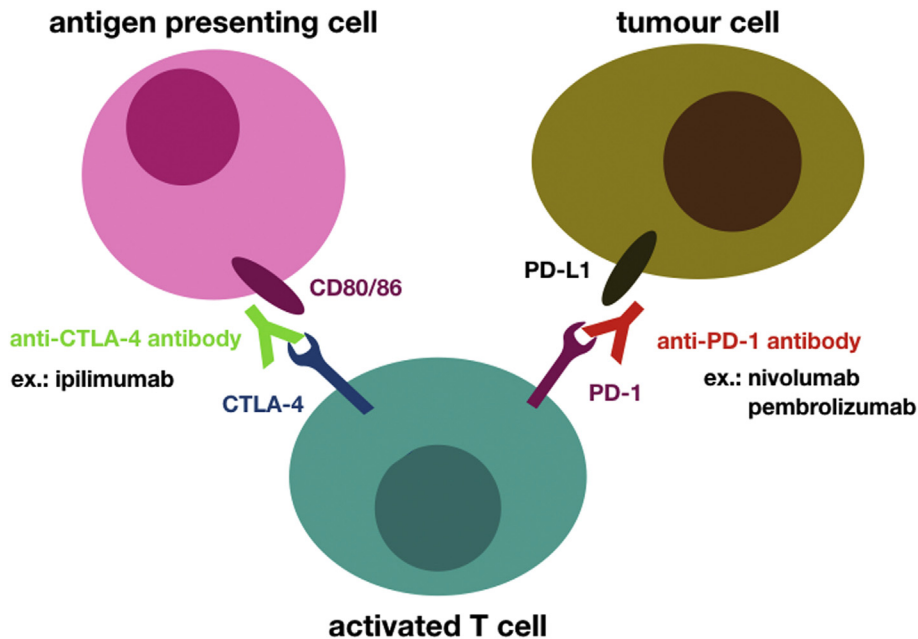


Fig. 1 How immune checkpoint inhibitors keep T cells awake. Engineered antibodies against CTLA-4 and PD-1, expressed at the surface of T cells prevent inactivation of the latter by ligands located at the surface of tumour cells and other immune cells in order to down-regulate T cell activity, allowing the immune system to mount an efficient T cell response against the tumour.

cancer research and therapy, decided to honour the two awardees and their findings with a short but very informative review in this issue of the Biomedical Journal [4].

First, Huang and Chang recapitulate the history and key discoveries of the two laureates, stressing the time, perseverance, and patience required to complete the long and trying road from bench to bedside, fuelled by their enthusiasm for, and dedication to scientific research.

Honjo, for example, came across PD-1 in apoptotic T cells in 1992 [5] and observed its role as a negative regulator of B cells, and its absence as the cause of autoimmune diseases in the late 90s [6,7]. As for CTLA-4, the immunoglobulin had been identified in 1987 by the group of Pierre Golstein [8]. In 1995, mice lacking CTLA-4 were found to display lymphoproliferative disorders and early lethality [9,10]. The same year, Matthew Krummel and James Allison showed that CD28 and CTLA-4 have opposing effects on T cell responses to stimulation, with CD28 being activatory, and CTLA-4 inhibitory [11].

Foreshadowing their Nobel Prize, both researchers were awarded the first Tang Award in Biopharmaceutical Science in 2014 [12]. The Taiwanese entrepreneur Samuel Yin created the prize in order to recognise and reward efforts in research fields essential to the 21st century, taking into account both technological progress and ethical considerations. Of note, the 2018 Tang Award of the same category went also to the field of cancer therapy, more specifically to the development of tyrosine kinase inhibitors for targeted cancer treatments, and was recently covered by another review in the Biomedical Journal [13].

Subsequently, the authors explain in detail the current applications of anti-PD-1 and anti-CTLA-4 drugs, separately or in combination, and to what extent these treatments have improved patient survival. The most outstanding example is

the case of advanced melanoma: last year, a phase 3 trial on a four year outcome study reported an over 40% survival rate for patients being treated with a combination of nivolumab (an anti-PD-1 monoclonal antibody) and ipilimumab (an anti-CTLA-4 monoclonal antibody). This is rather impressive, given that the survival rate had reached barely 10% so far [14,15].

The remarkable success of this trial has inspired a great range of further clinical trials for many types of cancer, including for the treatment of earlier stages of the disease. Huang and Chang complement their review with extensive and complete documentation on all important ongoing trials for immune checkpoint inhibitors in cancer. One important milestone for the application of anti-PD-1 medication was the US Food and Drug Administration (FDA) approval for the tumour-agnostic indication of pembrolizumab (another anti-PD-1 monoclonal antibody) for any kind of solid tumour displaying high levels of microsatellite instability (MSI-H) and deficient mismatch repair (dMMR) [16], the very first case of an anti-cancer drug to be granted this level of freedom.

Ultimately, the authors provide some perspective complementing the overall optimism, touching on the side effects of immune drugs, which are of variable severity. Another limitation to date is the fact that only a minority of patients benefits from this type of treatment. CAR T cell therapy, for instance, is so far limited to a restricted portion of patients with B cell malignancies [2]. Taking the discussion a little further, Demaria et al. point out in another recent update on cancer immunotherapy that tumour immunity is massively T-cell-centred, despite the fact that innate immune responses are crucial premises for efficient and lasting T-cell activities. Antigen-presenting cells, such as certain dendritic cells, are required for triggering the activation of tumour-specific CD8 T

cells, for example, by cross-presentation of tumour antigens. Natural killer (NK) cells in turn can recognise tumour cells and attack them directly, but also stimulate an adaptive response through the secretion of cytokines and growth factors. Tumours counter these attacks with inhibitory cell surface receptors, a fact that stresses that the fitness of the innate immune system should be taken into account as much as the adaptive one, and the range of target tumours massively increased [1].

Spotlight on original articles

From drug to death – deciphering the interactome of microtubule-targeting agents in prostate cancer

The incredibly fast-paced progress of techniques and technologies has radically changed our approach to decipher molecular pathways. For the most part, the performance, speed and accessibility of deep sequencing account for the gigantic zoom-out on networks, shifting the focus from the interaction between a few molecules to a class of molecules in its entirety, from proteome to epitranscriptome [17]. Knowing the potential chain reactions on the often strongly altered interactomes of cancer cells is of crucial importance in order to predict the (in)efficiency of specific treatments or the combined effect of several drugs.

By way of illustration, although the main targets of the two anti-cancer drugs Paclitaxel and Vincristine are rather well known, their secondary targets remain elusive, as do the details of their combined action, raising concerns pertaining to potentially unwanted secondary effects.

At the heart of this study by Delgado-Carreño and Méndez-Callejas stands the *in silico* identification and experimental validation of the main interactome nodes of these two drugs, with a special focus on secondary targets among the pro- and anti-apoptotic proteins [18].

The authors start with a comprehensive description of prostate cancer features, notably the issue that most patients with metastatic prostate cancer sooner or later become independent of androgen, and thus develop a resistance against androgen ablation therapy (ADT). This is where more drastic drugs become relevant, such as microtubule-targeting agents (MTAs). Allegedly, these naturally occurring substances caused the death of numerous hungry or bored hearse horses that nibbled at the leaves of the yew bushes surrounding Parisian cemeteries [19]. Paclitaxel, sold as Taxol, initially stems from the Pacific yew, while Vincristine, sold as Oncovin, is found in the Madagascar periwinkle. However, given that none of these substances can tell apart good from bad microtubules, the side effects of MTAs are rather severe, ranging from hair loss, to bleeding, and neuropathies [20], which explains why these treatments are only prescribed once more targeted methods like ADT fail.

Nevertheless, their impact is efficient. Delgado-Carreño and Méndez-Callejas briefly describe the molecular mechanisms of the microtubule-stabilizing agent Paclitaxel and the microtubule-destabilizing Vincristine. Some of the cornerstones of the process are the inactivation by phosphorylation of the anti-apoptotic Bcl-2 protein, the accumulation of the

pro-apoptotic protein Bim, and the induction of apoptosis through caspase activation [21].

The first step of deciphering further the Paclitaxel-Vincristine interactome is a bioinformatic one. The authors undertake a thorough literature based research and employ online tools to begin by identifying the essential nodes in the target network of each drug separately. They note that the Paclitaxel network presents a much higher number of nodes and edges than the Vincristine network. The former comprises TP53, AKT1, VEGFA, JUN and CASP3 as essential nodes, alongside BCL2, BAX and BCL2L1. Vincristine however, is principally centred on TP53 and CASP3, which are also part of the Paclitaxel network.

The investigation subsequently transits to the bench, where the effects of the drugs on cell proliferation and the induction of major cellular damage is confirmed in the androgen-independent prostate cancer cell line PC-3, which also carries a P53 mutation. Unsurprisingly, the cells are more sensitive to Paclitaxel than to Vincristine.

Further experimental quantification of protein levels based on the predicted nodes shows concomitant increase of Bim and decrease of Bcl-2 by both substances, but diverging effects on Bax proteins. Their levels are decreased by Paclitaxel and increased by Vincristine, while the effect on activated caspase 3 is the opposite.

Moreover, Delgado-Carreño et al. demonstrate that the direct interactions between the main players mediating the effect of the drugs differ significantly. For example, the Bim/Bax and Bcl-2/Bax levels increased and decreased respectively only after Paclitaxel treatment. In the case of the cellular response to Vincristine, new interactions are uncovered, notably between Bim and cleaved caspase 3, and the latter with Bcl-2. Time is also considered to be an important feature of the treatment course.

The authors conclude that although the Paclitaxel spectrum of action is much broader than the one of Vincristine, it harbours the risk of conflicting effects on pro- and anti-apoptotic pathways, while Vincristine is more specific for cell death induction. Their observations confirm previous results on Bim acting as a tumour suppressor, which takes over from the no longer functional P53 in promoting Bax release. Interestingly, Vincristine induces higher Bim levels than Paclitaxel.

Besides the confirmation that the time-course, the protein levels, and the exact modalities of apoptosis depend on the drug mechanism, Delgado-Carreño and Méndez-Callejas add valuable experimental information regarding predicted secondary targets of two MTAs in ADT-resistant, P53 negative prostate cancer cells.

Also in this issue

Original articles

The potential synergy of natural phenolic acids and hypoglycemic drugs.

The use of natural products, notably plant extracts, for adjunctive therapies is rapidly gaining popularity as well as some rigorous scientific support. Curcumin, green tea, garlic

and many other classics have made their way from the kitchen shelf to the medicine cabinet, and have proven their value against a broad range of diseases, such as cancer [22], tuberculosis [23] or *Helicobacter pylori* infection [24]. Aside from their intrinsic beneficial properties, natural compounds can display synergistic effects with medication, either enhancing the drug's impact or dampening detrimental side effects [25]. Phenolic acids and polyphenols are regulars among the active compounds in charge of antioxidative, antidiabetic [26], or neuroprotective effects [27]. On these grounds, Oboh et al. investigate in the present study [28] the influence of gallic and tannic acid on the therapeutic properties of the antidiabetic drug acarbose [29] in a rather surprising model system, the fruit fly, whose metabolic functional similarities with mammals are often underestimated [30]. The authors show that although the addition of the phenolic derivatives enhance the antioxidant and alpha-glucosidase inhibitory features of acarbose *in vitro*, they do not significantly improve the glucose lowering potential of acarbose *in vivo*, but displayed hypoglycemic effects on their own and supplementary antioxidant qualities when combined with acarbose.

Monitoring of Schneiderian membranes between tooth extraction and sinus lifting implant surgery by cone-beam computed tomography

Tooth extraction has made quite the progress since the countless vivid depictions from the Middle Ages of screaming patients with huge pliers stuck in their mouths, when the procedure was performed by lay barbers [31]. The same goes for the replacement of the lost tooth by implants, although the practice can be traced back to the Egyptians [31]. Nowadays tooth implantation is precision work, often requiring preliminary restoration of the jaw, due to bone atrophy and other complications. Additionally, the dentist's work is frequently impacted by the state and potential diseases of the maxillary sinus, neighbouring the oral cavity, which can affect the diagnosis and treatment options [32]. Detailed presurgical assessment of the patient's anatomy is thus a basic requirement. Notably the integrity of the Schneiderian membrane, the epithelium lining the maxillary cavity, ought to be preserved. Here, Hsu et al. perform a retrospective study on the correlation of the sinus membrane's thickness with the surrounding hard tissue next to a missing tooth, as well as the evolution of infected membranes after tooth extraction [33]. The authors don't detect a link between membrane thickness and bone features, and observe resolution of infection-caused thickening after an average of three months. Moreover, they confirm that the use of cone-beam computed tomography reduces the risk of damaging the Schneiderian membrane during subsequent surgery, and advise to wait about three months before performing sinus lifting implant surgery if an odontogenic tooth infection was present.

Important considerations for salvage liver transplantation following recurrent hepatocellular carcinoma

Hepatocellular carcinoma (HCC) occupies the infamous fourth rank of deaths by cancer, according to the World Health Organisation, and its incidence is increasing worldwide [34]. Treatment options drastically decrease with advanced stages of HCC, and prognosis is relatively poor [35]. Even the drastic

method of liver resection (LR) has a very high postoperative recurrence rate. So far, the best option for these patients consists in salvage liver transplantation (LT), but this option is hampered by the limited availability of donor organs, economic burden, risk of the operation, and the need of life-long immunosuppressive measures [36]. Thus, determining the combination of factors for an optimal outcome of LT is crucial for the improvement of therapeutic strategies.

For this reason, Chan et al. review the evolution of patients who had been subjected to liver transplantation for recurrent HCC after primary liver resection, and search for characteristics correlated with patient outcome in order to improve the decision-making process for HCC therapies [37].

They find that a maximum tumour size over 5 cm at initial resection, the exceedance of the UCSF criteria, and a period of more than a year between post-LR HCC recurrence and LT are independent risk factors for HCC recurrence after transplantation. Therefore, advanced HCC can affect patient outcome even after full replacement of the organ and LT should be carried out as soon as possible in case of HCC recurrence after resection.

Bismuth shields can negatively impact on computed tomography image quality

The 1979 Nobel Prize of Physiology and Medicine went to Allan Cormack and Godfrey Hounsfield for the development of computer assisted tomography, a method consisting in the computer-processed assembly of multiple X-ray images into virtual cross-sections. Nowadays, the "CT scan" technique is an essential tool for medical imaging, and used for countless purposes, including the detection of infarction, coronary artery disease, or complex fractures. However, the benefits come with a cost, such as the carcinogenic potential of the radiation [38], or damages due to intravenous contrast [39]. Bismuth shields could reduce the radiation dose to sensitive body parts, such as the eyes, but can interfere with signal acquisition. In order to assess the impact of bismuth shields on scan quality depending on the distance between the region of interest and the shield, Liao et al. performed a rigorous study on a phantom mimicking various tissues [40]. The authors conclude that bismuth shields can increasingly change CT number accuracy and induce artefacts the closer they are to the scanned part. They advise to limit their use to routine CT scans where the radiosensitive organs are not close to the diagnostic region and to avoid them in quantification-purposed or follow-up scans.

Warning headache foretells better survival after aneurismal subarachnoid haemorrhage

Subarachnoid haemorrhage (SAH), the bleeding into the two inner membranes of the meninges enveloping the brain and the spinal cord, is predominantly caused by severe trauma to the head. Spontaneous SAH cases in turn are due to the rupture of an intracranial aneurysm (aSAH) in 85% of all cases. Although the survival rates have substantially increased over the recent years, permanent cognitive impairments are common sequela [41]. Many warning symptoms have been noted, including "thunderclap" headache, seizures, vomiting [42], and even toothache [43], however none of them are very specific for aSAH. Nevertheless, a better knowledge on how

symptoms upon admittance at the emergency room correlate with clinical prognosis might help subsequent patient management, argue Lin et al. [44]. Their retrospective study shows that among several characteristics that can predict survival chances, the WFNS scale and the warning headache were the most predictive. Paradoxically, the headache seems actually to be a good sign, as it is associated with better survival rates, and should thus be taken into account for prognostic prediction.

Zirconia implant abutments display very good long-term performances

Implant abutments are the adapters connecting the dental implant, screwed into the bone, to a crown, bridge, or removable denture. They were traditionally made out of various metals, such as titanium, stainless steel, or gold [45].

More recently, zirconium has become a popular alternative, as the transition metal is both very resistant to corrosion and does not lead to the unattractive discoloration of the peri-implant soft tissue. Nevertheless, reports on medium- and long-term performance on zirconia implant abutments remain rare [46].

Here, Chen et al. review the state of 32 zirconia abutments and all-ceramic crowns six years after implantation [47] and observe that they display a 100% survival rate, good biological compatibility, and outstanding aesthetic results.

Conflicts of interest

The author declares no conflicts of interest.

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