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Oncolytic immunotherapy: an emerging new modality for the treatment of cancer

Cancer vaccines and immunotherapy have a long, often checkered history. This applies to the use of replicative ('oncolytic') viruses too. In each case, following initial excitement and a flurry of activity, the anticipated promise was not immediately realized and other than for a few stalwarts, each was largely discounted as a viable means of treating cancer. To some extent, those remaining active in these fields were seen as eccentrics who would not give up on ideas that had, unsuccessfully, had their day. To that extent, cancer vaccines and immunotherapies and oncolytic viruses have tracked each other in the ebb and flow of expectation and disappointment followed by the ultimate successful demonstration of clinical efficacy. In each case, I believe, this is likely in the infancy of what can be achieved. As ultimately the stories intersect, a brief discussion of each is warranted.

The concept of using the immune system to fight cancer began in the early 1900s when Robert Ehrlich postulated that cancer would occur at 'incredible frequency' if host defenses did not prevent the growth of continuously arising cancer cells, where 'immune substances … in the manner of magic bullets seek out the enemy'. This was followed in the 1940s by the observation that vaccination with tumor cells protected against chemically induced tumors in mice, and the discovery of T cells by Thomas and Burnett in the 1950s as 'the sentinels of the immune system' whose primary function was, they suggested, 'to protect against neoploastic disease'. From this followed the concept of cancer immunosurveillance. However, the discovery that immune compromised SCID mice were not more susceptible than normal mice to cancer in the 1970s threw the field into disarray, and most scientists discounted the possibility that the immune system could be used to fight cancer. This changed in 2001 when more fully immune compromised RAG2−/− mice were found to be more susceptible after all, which reinvigorated the field. From the huge volume of work conducted since the early days over 25 years ago, it is now possible to draw broad brush conclusions as to the likely requirements for effective immune-based cancer therapies.

So, what does not appear to work? While beyond the scope of this article to discuss in detail, small or uncontrolled studies have often shown initial promise with a variety of approaches, including rare clinical responses and/or apparently improved time to progression or survival. However, where definitive clinical testing has been undertaken (i.e. appropriately powered controlled phase III studies), this has pretty conclusively demonstrated that defined tumor antigen vaccines do not lead to improved overall survival in cancer patients. Likewise, in a definitive setting, cancer vaccines based on tumor cell lines, with or without additional cytokines, intended to contain a much broader complement of tumor antigens also appear to be ineffective for treating cancer. This is again even though some promising earlier results have been seen, including in combination with CTLA-4 blockade [1]. Approaches which have demonstrated promising or proven clinical efficacy were, early on, the use of irradiated autologous tumor cell suspensions derived from biopsies expressing GM-CSF as vaccines (GVAX; discontinued by Cell Genesys as not practical [2]), and more recently autologous cell-based approaches and immune checkpoint blockade. The cell-based approaches, as for autologous GVAX, suffer from considerable problems of practicality due to the patient-specific procedures and manufacturing required. In the case of T cells programmed to target a single tumor antigen, the durability of the effects seen may prove to be problematic (i.e. due to immune escape; data to date are mixed in this regard), and as such, the kinetics of response might be expected to be more reminiscent of a single-agent targeted therapy (initial deep response, followed by resistant clone outgrowth) rather than an immunotherapy where a sustained response is now expected to be seen.

Immune coinhibitory pathway blockade takes an alternative approach, that is, rather than the induction of a tumor-targeted immune response, the 'removal of the brakes' from tumor-targeted immune responses which may already be present in the patient by interrupting the mechanisms usually employed to protect against autoimmunity. As so far demonstrated for CTLA-4 or PD-1/PD-L1 blockade, this can give durable clinical responses in a proportion of patients. This is far more practical than the autologous approaches which have otherwise seen success, but side- effects can also be problematical (largely due to the induction of off-target autoimmunity, particularly when these agents are used in combination). Initially, it was thought that immune coinhibitory pathway blockade functioned by 'releasing the brakes' on tumor-targeted immune responses which could easily be measured, i.e. including common tumor antigens to which vaccines had previously unsuccessfully been made. However, while some correlation has been seen (e.g. [3]), attempts to measure these responses were not generally successful, and for some years, the actual mechanism remained unknown. While more complex than this brief discussion allows, and the details differ between the immune checkpoint targeted, it has now become broadly clear that not only does the efficacy of immune checkpoint blockade require a pre-existing antitumor immune response, but also this response must be to antigens uniquely present in the tumor when compared with normal tissue (i.e. to tumor neoantigens), rather than to 'traditional' tumor antigens (which are self-antigens) [4]. It has also become apparent that tumors themselves must display an

'inflamed microenvironment', i.e. contain infiltrating CD8 T cells and other markers of inflammation, for efficacy to result [5, 6]. The realization that immune responses to neoantigens are the key effectors of checkpoint blockade has led to considerable interest in the use of neoantigens for the manufacture of vaccines. The problem here is that the relevant neoantigens are specific to each patient, and therefore patient-specific procedures (biopsies), knowledge (resulting from deep sequencing), and manufacturing, are required. It is also not clear how to best translate that knowledge into actual vaccines. The general approach being applied appears to be the use of patient-specific neoantigen derived peptide vaccination, where it is not clear if this can generate a sufficiently potent immune response for efficacy. Identification of neoantigens which may be common across patients is also being pursued (i.e. patient-specific cocktails of pre-made commonly found peptides would be used), as are other vaccination approaches, e.g. RNA-based approaches.

A key question is therefore, what is the best and most practical way to initiate an anti-tumor neoantigen immune response, which may then be combined with immune coinhibitory pathway blockade (and potentially immune costimulatory pathway activation) to maximize the potency achieved.

This is where oncolytic therapy comes into play. Various attributes of cancer cells make them particularly sensitive to viral infection and replication. Indeed, the very characteristics of a cell which are needed to render it tumorigenic also tend to render it particularly sensitive to viral infection and replication. Viruses were initially tested for treating cancer in the early twentieth century following observations of spontaneous tumor remissions associated with natural virus infections. A hiatus then largely occurred until the 1990s when mutant viruses were used for the first time, using herpes simplex virus and adenovirus [7–11], but while safety was demonstrated, efficacy was limited. At that time, oncolytic viruses were largely thought of as direct cytotoxic agents. Viruses are, however, also extremely effective means to activate the innate arm of the immune system, i.e. to induce inflammation associated with virus infection, through pathways including cGAS/STING (which detects cytoplasmic DNA) and other pathogen-associated pattern recognition systems resulting in type 1 interferon production, and increased expression of MHC molecules, immune costimulatory ligands etc. Lytic virus replication also results in the release of various cellular component and cell fragments, which also results in damage-associated pattern recognition and inflammation, including inflammatory cytokine production, and the tumor and viral antigens released into this already highly inflamed milieu result in the induction of adaptive immunity as well. Preclinical data demonstrate that this includes the generation of immune responses to tumor neoantigens to which there were no immune responses before [12]. The efficacy of oncolytic viruses is, as a result, now known to include a substantial immune-based component, and the term 'oncolytic immunotherapy' has been coined. Indeed, it would seem unlikely that any other single approach could similarly activate both innate and adaptive immunity by such a combination of mechanisms, exactly as is needed for the activity of immune coinhibitory blockade to be effective, and also have the great benefit of clinical activity in its own right (rather than being merely an adjuvant or adjunct to other approaches). Importantly, oncolytic viruses are able to induce a patient-specific immune response to tumor neoantigens, in a fully off-the-shelf and practical product, without the requirement for any patient-specific procedures or knowledge. Second-generation oncolytic agents sought to more fully exploit the immune component of oncolytic therapy, in particular by using enhanced lytic potency viral backbones (such that more tumor antigen is released), combined with the expression of immune stimulatory cytokines such as GM-CSF [e.g. 13–15]. The first of these [talimogene laherparepvec (T-VEC); IMLYGIC, Amgen Inc.] was approved by the FDA in October 2015 (followed by EMA approval in December 2015) for the treatment of advanced melanoma following the observation of a 32% response rate (including 17% compete responses) and that these responses tended to be very durable in a 438 patient phase III trial [16, updated in 17]. While this was clearly an important milestone for the oncolytic virus field, there are now numerous treatment options for patients with melanoma, and it could be argued that efficacy across the entire study population was modest. In pre-specified subset analyses, however, first-line patients and patients without visceral disease (encompassing in each case ~50% of the enrolled study population) achieved greater benefit. Patients without visceral disease had a response rate of 40.5% and these and first-line patients demonstrated a substantial survival advantage too (HR of 0.57 and 0.50, respectively, both $P < 0.001$) [16, 18].

While single agent T-VEC is therefore effective for the treatment of melanoma, the real opportunity probably lies in the combination of oncolytic viruses with immune coinhibitory checkpoint blockade, based on the strong rationale described above, including the potential for oncolytic viruses to provide the left side of the equation (i.e. an anti-neoantigen immune response and inflamed tumor microenvironment) required for immune checkpoint blockade to be effective, i.e. to actually have something from which to 'release the brakes'. They may, therefore, be the perfect natural partners for combination, and potentially provide the 'golden combination' of immunooncology approaches. Preclinical data have borne this out with a number of oncolytic agents, showing synergy with immune checkpoint blockade in mice [19–21], and early clinical data with T-VEC have been very compelling indeed. Here, two phase Ib studies have been conducted with T-VEC combined with ipilimumab and pembrolizumab, respectively, both in advanced melanoma, and both showing a >50% response rate [22–24], higher than expected with any of the three agents alone, and without evidence of increased toxicity when compared with single-agent ipilimumab or pembrolizumab. The multiyear follow-up available in the case of the T-VEC + ipilimumab study shows these responses to be very durable too [22]. These data appear comparable with combined CTLA-4/PD-1 blockade, but with markedly reduced side-effects. Larger controlled studies (200 patient phase II study of T-VEC + ipilimumab [25]; 660 patient phase III study of T-VEC + pembrolizumab [26]) are now underway. Studies are now being extended into other tumor types too.

Overall, therefore, it seems likely that following a very long gestation (close to a century in each case), the use of the immune system and viruses to fight cancer are finally beginning to live up to the promise predicted by the early pioneers at the beginning of the twentieth century. Coincidentally (but unsurprisingly in hindsight), it appears that both share common

mechanisms of activity, and in particular likely exploit two sides of the same host defense-associated mechanism coin, providing truly synergistic complementary approaches. However, while the field of immunooncology is currently very high profile due to the remarkable recent clinical results achieved, it should not be forgotten that other of the 'newer' approaches can also provide very impressive clinical effects. This includes both single-agent and combination targeted therapies, such as in melanoma those targeting mutant BRAF or MEK (reviewed in [27]). It may well be the case that, particularly in cancer types which are less responsive to immune intervention, that combinations of targeted and immune-based approaches, together with in some cases traditional chemotherapy and radiotherapy, will be needed. However in which orders or combinations these different modalities should be used remains a matter of debate.

It is also the case that the clinical use of the only currently approved oncolytic agent, i.e. T-VEC, can provide some challenges in the clinic, including as T-VEC needs to be stored at −70°C or below, and pharmacies need to put in place procedures for handling this new agent. One would expect that future developments will include the use of formulations which are stable at higher temperatures, and broader experience will reduce the perceived challenges of implementation sometimes currently seen. T-VEC is also administered by direct intratumoral administration, and it is often suggested that intravenous administration would be preferable for an oncolytic agent. However, one has to remember that a key objective of oncolytic immunotherapy is to induce a very potent anti-tumor immune response. This seems unlikely to be achieved without also inducing a potent immune response to the virus. This immune response would preclude giving more than one or a very short course of effective intravenous virus doses before virus neutralization in the blood would occur. This is in addition to the problem of dilution of the virus dose in 5 l of human blood, very substantially reducing the effective virus concentration at the tumor site. While an extensive discussion of this area is beyond the scope of this article, I do not believe these challenges will be easy (or may be even possible) to overcome. There are no data to date which are convincing to me that they may be (either in preclinical studies or clinical trials). It is also the case that if the induced anti-tumor immune response is sufficiently potent, particularly when combined with other drugs, then intratumoral administration is not really a limitation at all (and may be generally advantageous in focusing immune effects on the tumor itself). If only a small number of tumors need to be injected, and only a limited number of times, then most tumor types should be able to be treated with oncolytic immunotherapy or combination oncolytic immunotherapy and immune checkpoint blockade, as the current approaches for taking biopsies can generally be used.

For the broader future of oncolytic immunotherapy, we can expect a rapid expansion of the number of clinical trials already being conducted combining this with immune coinhibitory pathway blockade, improved oncolytic agents being developed (i.e. with greater direct antitumor effects and with an improved ability to spread through tumors), and in particular the ability of viruses to deliver proteins directly to the tumor microenvironment to help further induce, enhance, and shape the antitumor immune response being exploited to a greater extent. The results of the first controlled trials of T-VEC combined with ipilimumab and with pembrolizumab (both currently underway as discussed above) will also be of great interest to the field.

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