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Review Article

Beyond checkpoint inhibition – Immunotherapeutical strategies in combination with radiation

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ABSTRACT

The revival of cancer immunotherapy has taken place with the clinical success of immune checkpoint inhibition. However, the spectrum of immunotherapeutic approaches is much broader encompassing T cell engaging strategies, tumour-specific vaccination, antibodies or immunocytokines. This review focuses on the immunological effects of irradiation and the evidence available on combination strategies with immunotherapy. The available data suggest great potential of combined treatments, yet also poses questions about dose, fractionation, timing and most promising multimodal strategies.

Contents

1. Immune checkpoint inhibition

The use of immune checkpoint inhibitors for the treatment of advanced melanoma [1] and other solid tumour entities [2–5] is establishing immunotherapy as a fourth pillar besides systemic anticancer treatments (conventional chemotherapy and targeted therapies), surgery and radiotherapy. Numerous clinical trials and preclinical projects have been started and CTLA4 (cytotoxic T-

Abbreviations: bsAb, bispecific antibody; CAR, chimeric antigen receptor; CDN, cyclic dinucleotides; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; GM-CSF, granulocyte-monocyte colony stimulating factor; IR, irradiation; PD-1, Programmed cell death protein 1 receptor; PD-L1, PD-1 ligand; scFv, single chain variable fragment; TCR, T cell receptor; Treg, regulatory T cells.

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lymphocyte-associated protein 4) and PD-1 (Programmed cell death protein 1 receptor)/PD-L1 (PD-1 ligand) antibodies have been FDA approved for the treatment of malignant melanoma and non-small cell lung cancer as well as other cancer types. The combination of immunotherapy with radiation is based on promising preclinical data and supported by a strong theoretical rationale [6–8]. To date, several clinical trials testing such combinations have been started for multiple cancer types [9] and the first results should be reported within the next years.

2. Immunotherapy beyond checkpoint inhibition

Yet, the spectrum of immunotherapy is much broader than immune checkpoint inhibition. Anti-tumour vaccination [10,11], cytokine based therapies [12], chimeric antigen receptor (CAR) T cells [13] and bispecific antibodies (bsAbs) [14,15] are only a few examples. Other strategies include toll-like receptor agonists [16], TGFβ blockade [17], NK cell based therapy [18] and immune modulation of macrophages [19] among others. Some of these strategies have been developed well before the clinical use of checkpoint inhibitors, yet with limited success. However, most of the clinical studies have been performed with immunotherapies as monotherapy, which leaves the question, if patients might benefit from combination therapies.

The last 2–3 decades have seen the development of two approaches that utilize polyclonal cytotoxic T lymphocytes (CTL) independently of T cell receptor (TCR)-mediated recognition of MHC (major histocompatibility complex) bound peptides for the elimination of tumours. Both, CAR T cells and bsAbs typically make use of a specificity-conferring single-chain variable fragment (scFv) derived from a monoclonal antibody to target a specific surface antigen on tumour cells. To generate CAR T cells, T cells are transduced with a recombinant fusion protein, in which such an scFv is fused to intracellular signalling components of the TCR and – at least in newer-generation CAR T cells – co-stimulatory domains usually derived from CD28 or 4-1BB are also incorporated [20]. T cell-recruiting bsAbs consist of two scFvs, one of which is directed against a tumour cell surface antigen. The other scFv is specific for the invariant CD3 signalling chain of the TCR and is able to recruit and activate tumour infiltrating T cells [21]. One of the biggest advantages of both approaches is the fact that every T cell, independent of its inherent specificity (through the α/β TCR), can be converted into a CTL for the specific lysis of tumour cells.

Vaccination strategies include "off-the-shelf" peptide vaccines as evaluated for renal cell carcinoma [22–24], personalized peptide vaccination approaches [25], as well as RNA vaccines [26] and strategies using dendritic cells [27] or whole inactivated tumour cells [28,29]. These therapies have the advantage of inducing tumour-specific immune responses targeting tumour-specific or tumour-associated antigens. Yet, vaccination as monotherapy, even in patients with minimal disease burden such as biochemical recurrence of prostate cancer after radical prostatovesiculectomy slowed PSA kinetics but did not control the disease in most patients [30,31]. The reason for that is most probably, that the tumour-inherent immunosuppression via Th2 polarization and intratumoural regulatory T cells (Tregs) do not allow the cytotoxic T cells primed by the vaccination to enter the tumour microenvironment [32] or exert their cytolytic function.

Cytokines have been established in oncological therapies for several years, e.g. IL-2 in melanoma [33,34]. Yet, systemic application can cause severe inflammation and even led to grade 5 toxicities in the case of IL-12 [35,36]. Therefore, recent efforts were focused on the development of tumour targeted cytokine application e.g. by coupling the active component with a tumour targeting antibody [37–40] creating so-called immunocytokines or complexing IL-2 with antibodies for altering binding specificities [41,42]. These therapies are able to overcome the general immunosuppression in the tumour microenvironment by converting the stroma into Th1 polarization, thus enabling T cells to enter the tumour and recognize their cognate antigens in context with co-stimulation on mature APCs. However, some cytokine effects are dependent on spatial distribution and exact concentrations. IL-2 is known to have dual effects depending on the concentration. It can either foster Th1 polarization and thus prime naïve T cells for anti-tumour responses or support Th2 polarization and Treg differentiation leading to a protumourigenic effect [43]. Thus, the effects of cytokine therapies might not be predictable and even heterogeneous in different patients and tumours depending on the tumour microenvironment.

3. Immune activation through tumour irradiation

During the last decade a paradigm shift has taken place acknowledging that besides the direct or indirect interaction of ionizing radiation with the radiosensitive DNA, secondary radiation responses additionally occur. In close proximity bystander effects and in distal sites of the irradiated area systemic, abscopal effects have been observed [44]. Distinct tumour cell death forms accompanied by the release of danger signals by IR-stressed cells and/or phenotypical cell alterations foster immune cell activation, thereby contributing to such non-DNA-targeted radiation-effects [7,45–47]. The so called immunogenic cancer cell death was originally linked to certain chemotherapeutic agents such as anthracyclines [48] and has been expanded to many stressors like radiation during the last years [49,50]. Characteristics and detection of immunogenic cancer cell death are discussed in the recently published consensus guidelines [51]. The key outcome is that tumour cells should be killed in a way that they become an intrinsic (*in situ*) cancer-specific vaccine and secrete danger signals to activate the innate immune system [7,52]. This can also be achieved by interfering with cell death pathways and consecutive induction of immunogenic necrosis [53].

The tumour microenvironment, too, can be modulated by radiation. Irradiation (IR) generates novel peptide sequences and enhances MHC class I expression [54]. Neoantigen-specific $CD8^+$ T cell responses have been shown to go along with tumour regression [55]. Radiation further enhances the diversity of the T-cell receptor repertoire of intratumoural T cells [56]. Some of the mutations that create neoantigens influence the response of patients to immune checkpoint inhibition. One pre-requisite for anti-tumour immune reactions is the infiltration of immune cells into the tumour tissue [57]. Neoadjuvant local IR with a single dose of 2 Gy causes inflammation and normalization of tumour vasculature and consecutively enables the recruitment of tumour-specific T cells. This was shown in the RIP1-Tag5 (RT5) transgenic mouse model expressing the simian virus 40 derived T antigen (Tag) as a model tumour antigen. M1 polarized macrophages in the tumour micro-milieu mediated the tumour infiltration of T cells by producing nitric oxide (NO) [58]. Currently, a randomized phase II study of radiation-induced immune boost in operable non-small cell lung cancer (RadImmune trial) evaluates the impact of low dose neoadjuvant irradiation in particular on $CD8^+$ T cell infiltration and secondarily on the association between $CD8^+$ T cell counts and progression free survival [59]. However, tumour irradiation has also been described to enhance tumour infiltration by Treg cells and immune system exhaustion [60] and to have a negative influence on anti-tumour immunity. In line with this, low dose IR can have anti-inflammatory effects including on macrophages [61], exploited for the treatment of benign, autoimmune T cell-driven inflammatory or degenerative diseases [62]. Additionally, the