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ABSTRACT

Hyperthermia holds great promise to advance immunotherapy in the treatment of cancer. Multiple trials have demonstrated benefit with the addition of hyperthermia to radiation or chemotherapy in the treatment of wide-ranging malignancies. Similarly, pre-clinical studies have demonstrated the ability of hyperthermia to enhance each of the 8 steps in the cancer-immunotherapy cycle including stimulation of tumor-specific immunity. While there has been an extensive recent focus on augmenting immunotherapy with radiation, surprisingly to date, there have been no clinical trials assessing the combination of hyperthermia with immunotherapy. The study of hyperthermia with immunotherapy is particularly compelling when considered in the context of a new treatment paradigm for this anti-neoplastic modality. Novel concepts include ease of treatment including elicitation of the tumor-specific response of not requiring whole tumor heating, potentially shorter treatment time, better treatment tolerance as opposed to other multi-agent approaches to immunotherapy and the ability to apply heat repeatedly with immunotherapies, unlike ionizing radiation. Several questions remained with regard to clinical integration which can be readily addressed with thoughtful clinical trial design building upon lessons learned at the bench and from clinical trials combining radiation and immunotherapy. Examples of promising avenues for clinical investigation of hyperthermia and immunotherapy including melanoma, bladder, and head and neck cancers are reviewed. In summary, there is a present convergence of factors in oncology that compel further investigation of the integration of hyperthermia with immunotherapy for the benefit of cancer patients.

Introduction

The ability of hyperthermia to augment radiation therapy and chemotherapy has been demonstrated in clinical oncology as evidenced in many phases II and III trials [1,2] While not yet assessed in clinical studies as is the case with radiation or chemotherapy, pre-clinical work strongly supports clinical investigation of hyperthermia in combination with immunotherapy. Hyperthermia stimulates a broad array of anti-neoplastic immune responses across the clinical therapeutic temperature range as demonstrated in numerous pre-clinical studies. Notably, hyperthermia has been shown *in vitro,* and in some cases, *in vivo* to favorably multiple steps of the cancer-immunity cycle [3]. These diverse mechanisms of action provide for a wide range of potential strategies to effect improved clinical outcome by way of the addition of hyperthermia to immunotherapy. While questions remain as to how best to integrate hyperthermia with immunotherapy, new thinking as to the application of hyperthermia in this setting provides a great opportunity to expand the use of both immunotherapy and hyperthermia to the benefit of cancer patients.

Hyperthermia – multifactorial immune effects

The effects of hyperthermia on the immune system are truly multifactorial as demonstrated *in vitro* and in some instances, *in vivo*. Hyperthermia results in both active and passive release of tumor antigens. At temperatures of $41–43\,^\circ C$ as commonly used in the clinic, HSP and tumor-specific cancer antigens can be released from intact cells in exosomes. Direct release of HSP and tumor antigen spillage can occur at these and higher temperatures [4,5]. Increased release of HSPs into the extracellular environment stimulates downstream immune activity and increases antigen presentation [6–8], thus promoting the beginning of the cancer immunity cycle. Once antigen uptake occurs, thermal stress facilitates the migration of antigen-presenting cells (APCs) to lymph nodes in part through up-regulation of MHC-I, MHC-II, and several co-stimulatory molecules (e.g., CD80, CD86, CD40) on APCs [9] with subsequent activation of T cells [10]. Furthermore, hyperthermia has been shown to enhance immune surveillance by T-cells [11] and also up-regulates the expression of toll-like receptor 4 (TLR4) on APCs, such as dendritic cells (DCs), and induces the release of cytokines,

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chemokines and nitric oxide which facilitate the induction of adaptive immune response [12]. The adaptive immune response includes some of the players of the innate counterpart, including T cells and APCs, and is equally heat sensitive. Heat, in addition, enhances T cell trafficking to tumor by controlling the persistence of lymphocytes by increasing the depletion of c-FLIP (a master anti-apoptotic regulator) [13] and inducing the expression of intercellular adhesion molecule 1 (ICAM-1) on high endothelial venules [11] which facilitate the trafficking of T cells to peripheral tissue. Hyperthermia also increases blood perfusion and decreases interstitial pressure in tumors, which may facilitate the infiltration of therapeutic co-stimulatory molecules or immune effector cells into tumors [14,15]. Migratory and cytolytic activity of NK [5] is also enhanced by hyperthermia by inducing the NKG2D clustering which recognizes MICA (MHC class I polypeptide related sequence A) on the tumor cell surface [16]. Finally, apoptosis of tumor cells results in part through thermal stress-induced up-regulation of Fas ligand (FasL) and cytokines in effector T cells [13]. Given hyperthermia can stimulate the immune system including tumor-specific responses, the addition of hyperthermia to immunotherapy is likely to augment clinical benefits obtained with immunotherapy alone.

Augmenting immunotherapy – a new paradigm for hyperthermia

The use of temperature for anti-neoplastic immune manipulation presents a distinctly new paradigm for the application of hyperthermia. The approach here proposed differs from the use of hyperthermia with radiation or chemotherapy in several key ways. Hyperthermia also has several advantages when compared to the use of radiation with immunotherapy that should facilitate clinical integration and expanded use of both immunotherapy and hyperthermia. Central concepts of this new paradigm include the lack of need to heat the entire tumor with the goal of eliciting tumor-specific responses, shorter treatment times, protracted repeated use, a favorable toxicity profile and potential for multifactorial immune effects

Partial tumor treatment

The use of hyperthermia to augment immunotherapy introduces a new paradigm for the application of heat in the therapeutic setting which addresses past barriers to its more widespread use. Hyperthermia has been primarily used to enhance radiation or chemotherapeutic effects. Numerous phase II and III trials have demonstrated the efficacy of hyperthermia when added to radiation or chemotherapy [1,2] yet hyperthermia remains an underutilized therapeutic modality in oncology. While utilization has increased in recent years, much of the resistance to use has been due to challenges in adequately heating the entire tumor as is required when combined with radiation therapy. Many studies have shown temperature achieved in all (T_{min}) or at least 90% of temperature sensors (T90) equate with better clinical outcomes [17–19]. The use of hyperthermia to augment

immunotherapy represents an important new paradigm for the application of hyperthermia that may significantly lower the bar for successful treatment. Stimulation of both tumor-specific and broad immune response with hyperthermia, may not require the entirety of the tumor be heated. It may be possible to stimulate a tumor-specific immune response by merely heating a portion of a malignant tumor which addresses one of the key challenges to the application of hyperthermia to enhance radiation or chemotherapy.

Reduced treatment time

While widespread practice calls for applying hyperthermia for one hour, it may be possible that significantly shorter treatment times are required to elicit desired immune responses. Simultaneous treatment, not routinely possible with radiation, may also be feasible. Given these considerations, should clinical hyperthermia be established as an effective immunotherapeutic agent, widespread adoption could be easily achieved for many tumors such as melanoma, head and neck and bladder cancer. Shorter treatment times combined with focal heating should facilitate patient convenience and acceptance.

Protracted repeated use

Current NCCN guidelines call for the use of checkpoint inhibitors including anti-PD-1 and anti-CTLA 4 therapy as first-line treatment of several malignancies such as metastatic melanoma and lung cancer. Patients responding to treatment receive an extended course of therapy, typically receiving treatment every 2–3 weeks over a period which frequently extends months or even years. Importantly, there are no limitations to repeated use of hyperthermia as opposed to ionizing radiation. Use of ionizing radiation is limited due to cumulative effects on normal tissues. Typically a short course of radiation is administered with a single cycle of immunotherapy after which responders continue treatment with immunotherapy alone. However, hyperthermia could be used routinely with each cycle of extended courses of anti-PD-1 therapy, anti-CTLA 4 or other immunotherapies as per standard practice, should benefit be established.

Favorable toxicity profile

Notably, hyperthermia is associated with only minimal toxicity as noted in dozens of clinical trials [1]. From the standpoint of toxicity, the combination of anti-PD-1 therapy with hyperthermia may be an attractive alternative to combined anti-PD-1 and anti-CTLA-4 therapy. While this combination therapy has been shown to improve relapse-free survival compared to either agent alone, this clinical benefit comes at the expense of grade 3 or 4 toxicity in approximately half of the patients, thereby limiting its use [20,21].

Multi-factorial immune effects

Hyperthermia has been shown to impact on multiple phases of cancer immunity cycle [3] as opposed to radiation for

which therapeutic strategies largely have been focused on individual steps. For instance, most of the focus with the use of radiation to stimulate immune response has been on tumor cell damage or death leading to tumor-specific antigen spillage with the development of an 'in situ' vaccine. Hyperthermia may similarly stimulate such response but may, for example, simultaneously stimulate NK cell activity and ICAM-1 up-regulation facilitating trafficking of t-cells primed for tumor destruction into the tumor micro-environment. It is noted that many of these concepts remain to be validated in vivo let alone in the clinical setting. This remains a challenge to be addressed as the application of hyperthermia with immunotherapy begins to be explored more broadly in actual therapeutic scenarios.

Questions to be addressed

There are several questions to be addressed in contemplating the addition of hyperthermia to immunotherapy. These questions include, sequencing with immunotherapy, specifics of timing, optimal thermal dose parameters for elicitation of specific immune responses, and identification of markers both prospective and post-treatment of response. Importantly, these questions can be readily addressed and answered building upon pre-clinical studies and clinical experience with hyperthermia and radiation with the effort put forth to date.

A basic principle to the administration of two or more cancer therapies is how to combine them maximizing safety and efficacy. From a safety standpoint, clinical experience with combined radiation and immunotherapy is likely applicable to a combination of heat and immunotherapy. Given the widespread use of immunotherapy, the issue of use with radiation arose early on in clinical integration. Published literature supports widespread clinical experience indicating that radiation and various regulatory approved immunotherapeutic agents can be safely combined [22]. The questions of sequencing and timing of radiation and immunotherapy to elicit optimal response have been more complex [23,24]. This question has been made more challenging by the limitations on the use of ionizing radiation. The ability to apply heat in varied timing to immunotherapy on an individual patient basis should facilitate treatment optimization. In the case of in situ vaccine strategy concerns with the destruction of immune cells critical for response by radiation whether in the tumor microenvironment or in lymph nodes should be of far less concern with hyperthermia, perhaps making timing less of an issue.

Thermal dose parameters – dependent on both temperature and time – equating with specific immune responses remain to be defined. Preclinical studies have demonstrated significant variations in how specific responses are elicited or augmented with heat. For instance, higher temperatures applied over a brief period may be best to augment CPI therapy [25] while trafficking of T cells to tumor may benefit from mild hyperthermia applied over a longer period of time [11]. Therefore, temperature gradients across a heated tumor may be helpful in eliciting wide ranging immunotherapeutic responses due to different dose profiles being associated with differing immune effects.

In recent years, there has been rapid clinical integration of genomic and mutational profiling to facilitate higher yield treatment selection. For instance, microsatellite or mismatched repair deficient (MMR) status now has a central role in selecting therapy for colorectal cancer. For patients with microsatellite unstable disease (MSI) or MMR deficiency, immunotherapy is the standard of care while for patients with microsatellite stable (MSS) disease, chemotherapy is standard [26]. In the case of CPI therapy, the role of PD-L1 expression as a marker for the effectiveness of hyperthermia will need to be defined. Lastly, genomic profiling may be useful in predicting which patients may benefit from hyperthermia in combination with immunotherapy in a similar way to a selection of radiation for the treatment of prostate cancer [27,28].

Promising areas for clinical investigation

Hyperthermia has the potential to enhance immunotherapy across a wide clinical spectrum. Promising avenues for initial clinical investigation include but are not limited to melanoma, bladder and head and neck cancers.

Melanoma

Clinical experience with hyperthermia to date
Melanoma is a disease for which benefit of hyperthermia has been established in a phase III trial as reported by Overgaard et al. In this multicenter trial, either 800 or 900 cGy for three fractions was administered locally to melanotic lesions with or without hyperthermia. Addition of hyperthermia was associated with significantly improved complete response rate of 46 vs. 29% with radiation alone with complete systemic and local control associated with improved survival with long-term follow-up [29]. While the investigation of application of hyperthermia alone with immunotherapy is of its own merit, it is interesting to note that the 800×3 regiment used in this trial has been found in a subsequent pre-clinical study in a mouse mammary tumor 1model to be the optimal radiation regimen to elicit abscopal responses [30]. This convergence of anti-tumor effects with this radiation regimen also used in the phase III melanoma trial raises intriguing questions as to how immunotherapy can be combined not only with hyperthermia but potentially in combination with both radiation and hyperthermia.

Clinical opportunities with immunotherapy
Melanoma has been the initial focus for the introduction of several classes of immunotherapeutics into the clinic. As a result, significant strides have been made in the treatment of melanoma with immunotherapy, yet there remains an acute clinical need for better outcomes. The first indication for a checkpoint inhibitor (CPI) was established in 2010 for the treatment of melanoma with ipilimumab, an anti-CLTA 4 agent [31]. Randomized clinical trials have subsequently

revealed a survival advantage with anti-PD-1 agents such as pembrolizumab and nivolumab as compared with the anti-CTLA-4 agent ipilimumab or chemotherapy [32–35]. Despite this progress, unfortunately only 30–40% experience any objective response and the median time to progression for those who do respond is between 5 and 7 months [32–35]. Complete response rates are considerably lower, generally in the range of 10–15%. Combined anti-PD-1 and anti-CTLA-4 therapy have yielded higher response rates in the range of 60% but at the cost of over half of patients experiencing grade 3 or 4 toxicity [20,21]. Due to the considerable toxicity of this treatment approach, a majority of the patients may not be suitable candidates for combined therapy due to age or comorbidities. In this large group of patients, single-agent anti-PD-1 therapy continues to be the preferred standard of care first-line option, despite the lower response rates. Therefore there remains a compelling need for therapeutic strategies to enhance existing immunotherapies for melanoma. As primary melanomas and many metastatic lesions are readily amenable to the use of superficial hyperthermia, application of hyperthermia can be done with relative ease and with repeated administration with each cycle of immunotherapy.

Bladder

Clinical experience with hyperthermia to date
Combined hyperthermia and chemotherapy has proven beneficial in the treatment of non-muscle invasive bladder cancer. In a multicenter trial, 83 patients with stage Ta andT1, gradeG1 to G3 transitional cell carcinoma of the bladder were randomized to receive mitomycin C with or without radiation therapy following complete transurethral resection. Patients with low-risk disease were excluded. Patient and tumor characteristics were evenly matched. Freedom from tumor recurrence, the primary endpoint was significantly improved with the addition of hyperthermia to 83% as compared to 42% with mitomycin C alone [36]. In a subsequent report of long-term results, this benefit was maintained with median follow-up for tumor-free patients of 91 months. Ten-year disease-free survival was 53% versus 15% with versus without hyperthermia [37].

Clinical opportunities with immunotherapy
Immunotherapy has an established role in the treatment of urothelial bladder cancer. Bacillis Calmette Geurin (BCG) was approved by the FDA in 1990 for intravesical treatment of superficial bladder cancer and remains the primary agent in the treatment of early bladder cancer to this day. Checkpoint inhibitor therapy is now part of the standard of care management for locally advanced and metastatic bladder cancer with an ongoing investigation of earlier use with chemotherapy in advanced disease. Atezolizumab, an anti-PD-L1 agent, was the first CPI approved by the FDA in bladder cancer for the treatment of locally advanced or metastatic disease refractory to platinum-based chemotherapy [38]. Atezolizumab administered every 3 weeks resulted in an objective response rate of 16 and 28% with robust PD-I1 expressing tumor-infiltrating immune cells [39,40] with respective 1 year overall survival of 37 and 50%, respectively. Presently, the combination of atezolizumab with or without gemcitabine and platinum-based chemotherapy is under active investigation. Similarly, other CPIs including nivolumab, pembrolizumab, avolumab and durvalumab are all in various phases of investigation and regulatory approval for treatment of bladder cancer. Other agents as, for instance, indolamine 2,3-dioxygenase (IDO) inhibitors and tumor necrosis factor receptor superfamily member 4 (OX40) targeted drugs are also under active investigation.

While results with immunotherapy are encouraging similar to melanoma, there is clearly an opportunity for improvement for which hyperthermia holds promise. The combination of immunotherapy and chemotherapy as assessed in ongoing trials presents an intriguing opportunity for the study of hyperthermia in combination with these two therapies. Beyond the potential enhanced immune effects, hyperthermia also enhances the effects of chemotherapy [41]. In particular, platinum-based agents have potential synergistic interaction with hyperthermia noting these agents cause DNA damage in tumor cells for which repair can be inhibited by hyperthermia [42]. Furthermore, in consideration of the different mechanisms by which immunotherapeutic agents now under study exert effects, hyperthermia may interact favorably in taking advantage of these varied immunotherapeutic strategies.

Head and neck

Clinical experience with hyperthermia to date
With regard to head and neck cancers, two randomized trials have revealed benefit with the addition of hyperthermia to radiation [43,44]. In one study, pre-dating routine use of chemotherapy in locally advanced head and neck cancer, patients were randomized to radiation alone versus radiation and hyperthermia. For patients with stage III or IV disease, the complete response rate was increased from 20 and 7% with radiation alone to 58 and 38%, respectively, with the addition of hyperthermia [43]. A phase III trial in Italy from this era assessed the impact of hyperthermia when applied with radiation in the treatment of N3 squamous cell cervical lymph nodes. A planned interim analysis inclusive of 41 patients revealed a complete response rate in the combined arm of 82% as compared to 37% with radiation alone. Long-term analysis revealed 5-year overall survival of 55 versus 0% in the hyperthermia versus radiation alone arms [44].

Clinical opportunities with immunotherapy
Similar to the other disease sites, immunotherapy now has an established role in the management of advanced and metastatic head and neck cancers, albeit with, at times, modest benefits as compared to other diseases. Cetuximab is a monoclonal antibody which targets the epidermal growth factor receptor (EGFR). Addition of Cetuximab to radiation is now standard treatment in locally advanced head and neck

cancer and similarly standard treatment, which is approved by the FDA for chemotherapy in recurrent or metastatic disease [45,46]. CPI therapy has subsequently been incorporated into the management of recurrent or metastatic disease. Pembrolizumab, regardless of PD-L1 expression, received accelerated approval by the FDA in 2016 following favorable results from a phase 1 b trial [47]. Overall response was 16% with a complete response of 5%. A subsequent phase III trial showed a 19% reduction in risk of death compared to chemotherapy which fell just short of statistical significance [48]. A subsequent study of first-line use of pembrolizumab compared with chemotherapy in recurrent or progressive disease revealed improved overall survival with immunotherapy [49]. Similar results have been noted with nivolumab including a phase III trial for patients with recurrent disease. This study showed a response rate for nivolumab of 13% and significant but clinically modest improvement in overall survival from 5.1 to 7.5 months with nivolumab vs. chemotherapy or cetuximab. Greater difference in one-year survival, however, was noted with nivolumab: 36 vs. 17% [50]. Current investigational efforts are focusing on the combination of immunotherapy with chemoradiation in the primary treatment of locally advanced disease.

There are several clinical opportunities to be explored for the combination of hyperthermia with immunotherapy in the treatment of head and neck cancers. Noting the generally modest response rates of CPIs for head and neck cancers as compared with melanoma, hyperthermia may play an important role in expanding efficacy and use in this patient population. Similar to and expanding upon opportunities with bladder cancer, a combination of hyperthermia not only with chemotherapy and immunotherapy but also radiation therapy may provide for multifactorial treatment enhancement with the addition of heat. Hyperthermia might also enhance monoclonal antibody expression for agents such as cetuximab in head and neck cancer, herceptin in breast cancer, and rituximab in non-Hodgkins lymphoma CD20. A collaborative study between the National Cancer Institute and Thomas Jefferson University found that radiation enhances total and cell surface expression of these monoclonal antibodies [51]. Preliminary analysis of hyperthermia revealed similar potential with increased tumor cell sensitivity to NK cell-mediated killing and antibody-dependent cell-mediated cytotoxicity with cetuximab.

Conclusions

Hyperthermia holds great potential to further the application of diverse approaches to immunotherapy to the benefit of cancer patients. Additional mechanistic and *in vivo* studies will aid in the realization of promising strategies for combining these two modalities. New paradigms applied to the application of hyperthermia with immunotherapy stand to address identified limitations to each of these modalities. Compelling clinical indications including use in the treatment of melanoma, bladder and head and neck cancers amongst others await clinical study well deserving of a fraction of resources dedicated to the study of other strategies for augmentation of immunotherapy.

Disclosure statement

No potential conflict of interest was reported by the author.

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