Clinical Cancer Research

For Whom the Cell Tolls? Intratumoral Treatment Links Innate and Adaptive Immunity

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Intratumoral immunotherapy can potentially modulate the tumor microenvironment (TME) and potentiate the effects of concomitant or sequential systemic immunotherapies. Intratumoral administration of different Toll-like receptor agonists, including TLR4, can potentiate these effects through innate and adaptive immunity connection.

See related article by Bhatia et al., p. 1185

In this issue of *Clinical Cancer Research*, Bhatia and colleagues (1) report the first human clinical trial for the intratumoral administration of the Toll-like receptor (TLR) type IV agonist G100 in Merkel cell carcinoma (MCC). This molecule is a fully synthetic analogue of lipopolysaccharide (LPS). Ten patients with injectable lesions from MCC (both potentially resectable and metastatic) were treated with 5-μg G100 via intratumoral administration for 1 and 8 days (for patients with resectable tumors, $N = 3$) before radical treatment, or for 1, 8, and 22 days up to 3 cycles (for patients with unresectable tumors, $N = 7$). Patients often experienced mild local toxicity, but no serious adverse events were observed.

In terms of efficacy, 2 of 3 patients with resectable tumors (1/3 experienced a complete pathologic response) were disease-free for more than 3 years. Two of 7 patients with metastatic tumors had objective responses for more than 2 years. One patient had been previously treated with surgery alone, and the other with chemotherapy, radiotherapy, and multiple immune checkpoint inhibitors, including an anti-CD137 agonist antibody.

The authors also explored in detail the tumor microenvironment (TME) effects of G100 in posttreatment biopsies, revealing that patients who benefited were those with increases in CD4 and CD8 T-cell infiltration. However, gene expression profiling did not show differences between responders and nonresponders. For responders, increases in the expression of genes related to macrophages, dendritic cells, and T-cell activation were observed, perhaps indicating the activation of both innate and adaptive immunities in the TME. In peripheral blood, a significant increase in the diversity of TCR clones among responders was observed, likely indicating broader antitumor reactivity. This finding supports the concept that even if acting locally, systemic immune effects do occur.

Furthermore, the study by Bhatia and colleagues provides translational and clinical evidence for the emerging role of intratumoral strategies in treatments for metastatic or localized solid tumors. The rationale of this approach is as follows: first, acting in the TME and tumor-draining lymph nodes turns on innate and adaptive immune cells and other stromal components that may recirculate (2); second, it is safer because of limiting systemic exposure that otherwise would be toxic, as observed with LPS in septic shock.

With these ideas in mind, multiple types of intratumoral treatments can be deployed, comprising from modified viruses and bacteria to synthetic compounds or antibodies, for an *in situ* vaccination effect that could turn a "cold" immune-negligent tumor into a "hot" immune-active tumor. Bacillus Calmette–Guérin vaccine used for localized urothelial carcinoma, and imiquimod (TLR7 agonist), or more recently, T-VEC for melanoma, are examples of local oncology therapies available in the clinic (2).

Pathogen-associated molecular patterns (PAMP) activate the innate immune system through various mechanisms that are being explored in several clinical trials. In addition to surface or endosomal TLRs, other PAMP receptors are currently being developed as therapeutic targets, including melanoma differentiation–associated protein 5 (MDA-5), retinoic acid–inducible gene 1 (RIG-1), or the stimulator of IFN gene (STING; ref. 2).

The molecule used in this clinical trial, G100, is a TLR4 agonist. TLRs constitute a family of transmembrane receptors, with 10 different members described in humans. TLRs have different subcellular locations (cytoplasmic or endosomal membrane) and respond to specific ligands (Fig. 1). Ligands may come from foreign microorganisms (e.g., peptidoglycans, viral RNA, or LPS) or from endogenous sources. Endogenous TLR agonists are the so-called damage-associated molecular patterns (DAMP) that are released or activated following tissue damage or cell death. TLR4 is expressed on the plasma membrane of multiple leukocyte types and endothelial cells, and is not only activated by LPS as an exogenous ligand but also by HMGB-1, oxidized LDL, and a splicing form of fibronectin that occurs upon inflammation as endogenous ligands.

TLR-4 activation is linked to dendritic cell activation/maturation, and these leukocyte populations will ultimately be in charge of presenting tumors antigens to cytotoxic T cells (3). In this regard, chronic exposure may result in desensitization of the antigen-presenting system as observed upon systemic delivery of LPS. Local activation of TLRs in the TME by intratumoral

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doi: 10.1158/1078-0432.CCR-18-2690

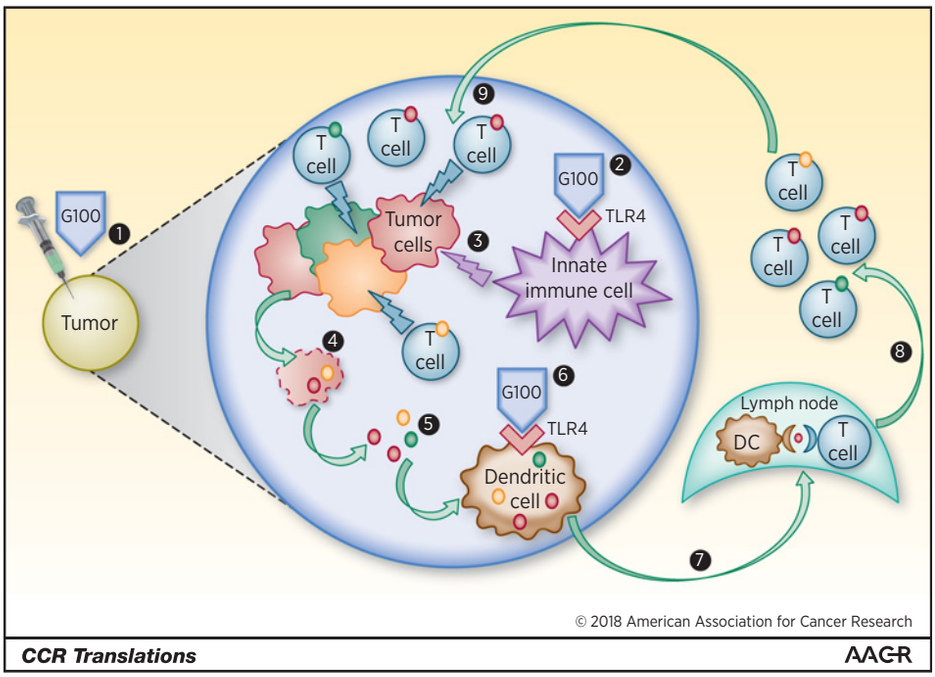

Figure 1.
Possible mechanism of action of G100 antitumoral activity. (1) G100 is injected in the tumor and (2) activates TLR4 in innate immune cells present in the tumor, which (3) attack tumor cells, producing (4) an immunogenic cell death, including the release of tumor antigens, which are in turn captured (5) by dendritic cells (DC). (6) TLR4 activation by G100 also activates and matures DCs, which (7) effectively cross-present cancer antigens to T cells. (8) T cells expand, (9) seek out, and destroy tumor cells, likely restarting the cycle and potentiating the connection between innate and adaptive immunity. In these settings, adaptation of the tumor is expected with immunosuppressive mechanisms such as PD-L1 expression, thus requiring combinatorial approaches.

treatment could create a second wave of signals that initiate more efficient immune responses against cancer, which could exert later a systemic and distant effect as a result of lymphocyte recirculation from the TME or tumor-draining lymph nodes (Fig. 1).

The clinical trial by Bhatia and colleagues uses a monotherapy approach that offers a proof-of-principle, but most likely will be insufficient by itself. Combinations of different local TLR agonists and multiple systemic treatment strategies have either been reported or are ongoing. Promising results for these combinations have been observed with TLR9 and TLR3 agonists.

TLR9 is located in endosomes and is activated by nonmethylated CpG DNA sequences, abundantly present in viral and bacterial DNA but much less so in eukaryotes. In patients with metastatic melanoma, who were resistant or naïve to anti PD-1 therapy, there is evidence for clinical activity for the combination of a local TLR9 agonist and anti-PD-1 mAb. CMP-001 and SD-101 are synthetic TLR9 agonists that have been combined with pembrolizumab. The first one achieved a 22% objective response rate (ORR) in patients with melanoma that were resistant to anti-PD-1 mAb (4), and the second one has shown a 78% ORR in anti-PD-1 naïve patients and 15% in anti PD-1 resistant (5). An increase in the expression of genes with an immune inflammatory signature was detected in both posttreatment biopsies.

TLR3 is also located in endosomes and binds to double-stranded RNA ligands that indicate viral infections. Synthetic poly I:C double-stranded RNA has been previously explored as an adjuvant in cancer therapy (2). Two derivatives of poly I:C are currently in clinical trials: Hiltonol and BO-112. Reported clinical experience with intratumoral delivery of Hiltonol (poly ICLC because polyC is stabilized by L-lysine) suggests clinical activity by stabilization of disease and proimmune effects in the TME (6). BO-112 is an improved version of poly I:C, nanoplexed with the polycationic carrier polyethylenimine, which avoids degradation by endonucleases, and confers activity not only through TLR3 activation, but also through RIG-1 and MDA-5, thus improving the antitumoral responses to anti-PD-L1 in preclinical models (Melero and colleagues; submitted for publication). In patients, IT administration of BO-112 has demonstrated a manageable safety profile, a direct antitumor activity, an increase in the number of inflammatory cells, and increased local expression of immune-related genes (7). This agent is currently being explored in combination with anti-PD-1 antibodies for PD-1–refractory patients (NCT02828098).

Overall, intratumoral TLR agonists are safe and attractive treatments to be explored, both as single agents and especially in combination with immune checkpoint inhibitors. Many aspects need to be investigated to fully exploit the potential of intratumoral TLR agonists. To name a few: (i) injections of more than one amenable lesion, (ii) its combination with radiotherapy/chemotherapy or other means to elicit immunogenic tumor cell death, and (iii) administration in surgical cases as a neoadjuvant treatment to prevent relapse. Expert consensus will be required to provide guidelines and standards on how to intratumorally administer and radiologically measure responses in locally injected and noninjected lesions (Brody and colleagues; submitted for publication). Reproducibility and reliability of local administration is not trivial, and eventually these strategies must be demonstrated for the prolongation of overall survival.

Disclosure of Potential Conflicts of Interest

I. Marquez-Rodas is a consultant/advisory board member for Bioncotech, Bristol-Myers Squibb, MSD, Novartis, Pierre Fabre, and Roche. A. Calles is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novartis, Pfizer, and Roche/Genentech. I. Melero reports receiving commercial research grants from Alligator, Bioncotech, Bristol-Myers Squibb, and Roche, speakers bureau honoraria from MSD, and is a consultant/advisory board member for

Alligator, Bayer, Bristol-Myers Squibb, F-Star, Genmab, Merck Serono, Roche, and Tusk. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

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Received September 3, 2018; revised September 12, 2018; accepted September 21, 2018; published first September 27, 2018.

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