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Endogenous and pharmacologic targeting of the STING pathway in cancer immunotherapy

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The past years have witnessed remarkable progress in the clinical efficacy of cancer immunotherapies, particularly with antibodies blocking the T cell inhibitory receptors CTLA-4 or PD-1 (1). However, these therapies are effective in only a subset of patients, and one of the primary mechanisms underlying the failure of these agents appears to be the lack of a T cell-inflamed tumor microenvironment at baseline (2). As such, new strategies to transform a non-T cell-inflamed tumor microenvironment into one that supports T cell-based inflammation are being pursued. One ideal approach would be to elicit an innate immune activation cascade within the tumor microenvironment sufficient to prime a new T cell response against tumor antigens and also support the recruitment of activated T cells into the tumor site. To this end, detailed investigation into the key innate immune pathways involved in natural tumor sensing has been carried out, with the aim of developing new pharmacologic approaches to trigger such pathways therapeutically.

Analysis of human tumors that do show spontaneous T cell infiltration revealed that CD8⁺ T cell infiltration was associated with a type I IFN transcriptional signature (3). Mouse mechanistic experiments demonstrated that animals deficient in type I IFN signaling showed defective priming of T cells against tumor-associated antigens (4, 5). These observations led to an investigation into the identity of the innate immune sensing pathways triggered in the presence of tumors in vivo that drive type I IFN production by host APCs. Mice deficient in TLR signaling, extracellular ATP sensing, or cytosolic RNA sensing showed no defect in spontaneous T cell activation in response to tumor challenge. However, mice deficient in the STING pathway involved in cytosolic DNA sensing showed blunted type I IFN production, reduced anti-tumor T cell priming, and failed rejection of immunogenic tumors (6). The STING pathway is activated in the presence of cytosolic DNA, which is sensed by cyclic-GMP-AMP synthase (cGAS), an enzyme that generates cyclic GMP-AMP (cGAMP), the endogenous ligand of STING (7). This results in the auto-phosphorylation of tank-binding kinase 1 (TBK1) and phosphorylation of the transcription factor interferon regulatory factor

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3 (IRF3), which activates the transcription of type I interferon (IFN) genes. Consistent with this model, tumor-infiltrating DCs were found to contain tumor-derived DNA within the cytosol, which correlated with translocation of IRF3 to the nucleus and expression of IFN- β . Thus, these data suggest that the STING pathway is the main innate immune pathway involved in sensing of tumors, and its endogenous activation in APCs leads to subsequent T cell priming against tumor-associated antigens. Interestingly, the adaptive immune response that contributes to tumor control upon radiation therapy was also shown to be dependent on host type I IFN signaling (8). Mechanistic studies revealed that activation of the STING pathway, but not TLR signaling, was required for the therapeutic effect of radiation in vivo (9). These data suggest that the form of cell death induced by radiation facilitates the delivery of DNA or STING ligands to host DCs, thus facilitating T cell priming.

The discovery of the importance of STING in innate immune sensing of tumors implied that deliberate stimulation of this pathway by pharmacologic means might boost these mechanisms further and have a therapeutic effect against tumors in vivo. With this rationale in mind, the development of direct agonists of STING for use as cancer therapeutic has become an area of intense investigation. Preclinical studies using the agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) showed potent anti-tumor activity in various mouse models. However, although its anti-tumor effect in mice was well established, this agent showed no effect in the clinic when combined with chemotherapy in a Phase III trial in non-small cell lung cancer. More recently, DMXAA was shown to directly interact with mouse STING and to induce cytokine production by APCs. Structure-function studies revealed that, due to differences in specific amino acid residues between mouse and human STING, DMXAA can only interact with the murine molecule. This fact likely explains the lack of clinical activity of this compound in human cancer patients, and prompted the development of new agents that might serve as direct human STING agonists. The realization that cyclic-dinucleotides (CDNs) are the direct natural ligands of STING facilitated the development of STING-activating therapeutics. At the same time, it has become apparent that the human TMEM173 (STING gene) is polymorphic, and natural CDNs do not stimulate all human STING variants. Thus, chemical modifications of CDNs were performed and synthetic dithio mixed-linkage CDNs were identified that showed the capacity to activate the five known human STING variants (10). The lead molecule ML RR-S2 CDA showed improved properties in vitro and in vivo. Therapeutically, intratumoral injection of this molecule resulted in impressive tumor regression in the B16 melanoma model, CT26 colon cancer model, or the 4T1 breast cancer model. In addition, a robust systemic antigen-specific CD8⁺ T cell response was induced that was sufficient to reject distant, non-injected tumors and conferred complete protection against tumor re-challenge. Thus, intratumoral STING agonists have emerged as a novel candidate treatment approach for clinical translation.

In summary, identification of the STING pathway in the tumor microenvironment as an important innate immune sensing mechanism that drives type I IFN production and subsequent priming of endogenous anti-tumor T cells has led to the pursuit of novel STING agonists as a cancer therapeutic. Moving towards clinical trials with human STING agonists should receive a high priority as a potential strategy for igniting de novo immune responses

in the setting of non-T cell-inflamed tumors. This approach could expand the fraction of patients potentially benefitting from immunotherapies.

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Figure.

Working model of innate immune sensing of tumors that leads to spontaneous T cell responses in vivo. The STING pathway is activated within intra-tumoral dendritic cells (DCs) by tumor-derived DNA. Recognition of cytosolic DNA by cyclic GMP-AMP (cGAMP) synthase (cGAS) leads to the generation of cGAMP, the endogenous ligand of STING (stimulator of interferon genes). Therapeutically, the STING pathway can be stimulated by direct STING agonists, when the compounds are injected into the tumor site. This results in the phosphorylation of tank-binding kinase 1 (TBK1) and the transcription factor interferon regulatory factor 3 (IRF3), which induces expression of the type I IFN genes. Type I IFN signaling in the BATF3 (basic leucine zipper transcription factor ATF-like 3)-lineage of DCs leads to antigen-specific T cell priming, generating spontaneous anti-tumor T cell responses in vivo. Recruitment of effector T cells into the tumor microenvironment is then facilitated by the release of CXCL9/10 chemokines from DCs and other cells at the tumor site. Successfully recruited activated T cells induce direct tumor cell killing, leading to measurable tumor shrinkage.