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STING agonists in cancer immunotherapy: a brief review

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Abstract. The “STimulator of INterferon Genes” (STING) represents the primary sensors of cytosolic double-stranded DNA (dsDNA). The STING cellular signaling pathway is considered an attractive pharmacological target for cancer immunotherapy due to its immunostimulatory potential. In fact, activation of the intracellular STING protein triggers the secretion of type I IFNs, which results in immune-mediated tumor elimination and generation of antitumor immune memory. Two types of STING agonists were developed: cyclic dinucleotides (CDNs) and non-nucleotide small molecule agonists. Preclinical studies of STING agonists have demonstrated remarkable results in many tumor models, resulting in complete and durable therapeutic responses in a majority of treated mice. This review provides a brief summary of the latest research findings on STING agonists, their delivery to the tumor and the strategies being employed to enhance their efficacy in cancer immunotherapy.

Keywords: Stimulator of INterferon Genes, STING agonists, type I interferon, antitumor response, drug delivery.

INTRODUCTION

The innate immune system is the first defense line in mammals. One of the significant immune responses is the pattern recognition receptors (PRRs), which recognize the different pathogen and damage-associated molecular patterns (PAMPs and DAMPs). PRRs are germline-encoded, and they play a crucial role in the immune system’s function (Hopfner *et al.*, 2020). PAMPs and DAMPs derived from bacteria, viruses, or endogenous cytosolic self-DNA from tumor cells activate PRRs, leading to the production of soluble mediators such as type I interferons and pro-inflammatory cytokines (Amouzegar *et al.*, 2021).

The “STimulator of INterferon Genes” (STING) family of PRRs was discovered in 2008, and they are considered the primary sensors of cytosolic

double-stranded DNA (dsDNA) (Ishikawa *et al.*, 2008). STING protein is expressed in both innate and adaptive immune cells, and non-immune cells, including endothelial cells, epithelial cells, and cancer cells (Garland *et al.*, 2022). STING activation has immunological effects that are mediated by the secretion of IFNs, especially type I IFNs (IFN-I) such as IFN- β . The therapeutic responses to STING activation include dendritic cell maturation, antitumor macrophage polarization, enhanced priming and activation of T cells, improved T cell infiltration in tumor sites, and promotion of natural killer (NK) cell activation (Garland *et al.*, 2022). These responses to the STING pathway activation result in immune-mediated tumor elimination and the generation of antitumor immune memory. Therefore, the STING pathway is considered an attractive pharmacological target for cancer immunotherapy due to its immunostimulatory potential. This review provides an overview of the latest research findings on STING agonists in cancer treatment. It also discusses their delivery to the tumor and the strategies being employed to enhance their efficacy in cancer immunotherapy.

STING CELLULAR SIGNALING PATHWAY

Typically, cytosolic nucleases break down foreign DNA in cells. However, if there is abnormal dsDNA in the cytosol due to pathogenic infections or cellular damage, this activates the cyclic guanosine monophosphate-adenosine monophosphate synthase cGAS (discovered by Sun *et al.* in 2013), which synthesizes a cyclic dinucleotide called cGAMP (2'-3'-cyclic GMP-AMP). This signals the STING protein in the endoplasmic reticulum, which moves to the perinuclear Golgi and binds the TANK-binding kinase 1 (TBK1). Together, the complex phosphorylates transcription factors like IRF3 and NF- κ B, which leads to the production of IFN-I in the cell.

STING AGONISTS IN CANCER IMMUNOTHERAPY

To achieve anti-cancer effects by mimicking the activation of STING pathway signaling, STING agonists have been developed. STING agonists come in two types: cyclic dinucleotides (CDNs) and non-nucleotide small molecule agonists. CDNs are the natural ligands of the STING protein but face limitations in clinical applications due to their chemical properties. Synthetic CDNs are being formulated to improve their performance, with ADU-S100 being tested in clinical trials for head and neck squamous cell carcinoma

and lymphomas (Zandberg *et al.*, 2020). Among the non-nucleotide small molecule agonists, dimeric amidobenzimidazole (diABZI) is one of the most promising. Discovered in 2018 by Ramanjulu *et al.*, diABZI is the first effective non-nucleotide STING agonist used worldwide, with great potential to enhance human cancer immunotherapy. In immunocompetent mice with syngeneic colorectal tumors (CT-26), intravenous injection of diABZI has demonstrated tumor regression and increased overall survival time. In fact, 80% of treated animals were tumor-free until the end of the study (Ramanjulu *et al.*, 2018).

DELIVERY AND COMBINATION STRATEGIES FOR STING AGONISTS

The STING protein is located on the endoplasmic reticulum. To interact with STING, CDNs STING agonists need to passively diffuse through the lipophilic plasma membrane, which is challenging due to their anionic phosphate groups and high aqueous solubility (Garland *et al.*, 2022).

Recent research shows that using liposomal delivery for STING agonists could have significant benefits. Different approaches are also in development: one study, led by Tse and colleagues, utilized lipid nanoparticles to deliver mRNA vaccines that encode a gain-of-function mutation of STING. This mutation allows for the expression of constitutively active STING, even without STING ligands. The researchers found that mice vaccinated with mRNA encoding STING^{V155M}, and inoculated with TC-1 tumors transformed with oncoproteins, experienced suppressed tumor growth and longer survival (Tse *et al.*, 2021).

A different team has created a cancer vaccine called STINGVAX that uses a CDNs STING agonist and granulocyte-macrophage colony-stimulating Factor (GM-CSF)-secreting cancer cells (Fu *et al.*, 2015). The study found that STINGVAX was able to successfully slow down the growth of tumors in mice with B16 melanoma, with just one injection. Tumors from mice who received STINGVAX had more CD8⁺ IFN- γ ⁺ T cells compared to mice who were given a cancer cell vaccine without CDNs.

Moreover, the idea of combining STING agonists with existing immunotherapies is quite attractive. While CTLA-4 and PD-L1 blockades can successfully revive faltered T-cell responses, they rely on the availability of T cells and entry to the tumor core. STING agonists may act as an on-site vaccine, stimulating the T-cell response and reducing the threshold of myelosuppression that causes immune exclusion.

CONCLUSIONS

Currently, a crucial goal in the field of immuno-oncology is to discover new immunotherapeutic approaches that boost the immune system's ability to recognize and eliminate tumors that do not respond to FDA-approved immune checkpoint inhibitor antibodies.

The STING cellular signaling pathway is highly promising in this regard. Since the discovery of the STING protein in 2008, academic interest in this area has grown exponentially, as evidenced by the rapidly increasing number of publications, and by the development of an expanding number of STING agonists.

Significant advancements have been made also to improve the delivery of STING ligands: nanoparticles improve cytosolic cellular uptake and increase local retention of CDNs, offering a suitable approach for addressing the pharmacological shortcomings of locally administered CDNs.

Preclinical studies of an increasing number of STING agonists have demonstrated remarkable results in many tumor models, sometimes resulting in complete and durable therapeutic responses in a majority of treated mice.

There are still many open-ended questions regarding STING agonist therapy in human patients at this time, but the immense potential justifies the large number of researchers engaged on the topic.

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