STING-driven activation of T cells: relevance for the adoptive cell therapy of cancer

Fabian Richter1,2, Christophe Paget1,2 and Lionel Apetoh3,*

1 Centre d’Étude des Pathologies Respiratoires, U1100, INSERM, Tours, France.
2 Faculté de Médecine, Université de Tours, Tours, France.
3 Brown Center for Immunotherapy, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA.
* Corresponding Author:
Lionel Apetoh, Brown Center for Immunotherapy, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA; E-mail: lapetoh@iu.edu

ABSTRACT Adoptive cell therapy (ACT) can successfully treat hematopoietic cancers but lacks efficacy against solid tumors. This is due to insufficient T cell infiltration, high tumor heterogeneity, frequent antigen loss with subsequent tumor escape, and the immunosuppressive tumor microenvironment (TME). Alternative methods to boost the anticancer efficacy of adoptively transferred cells are actively pursued. Among adjuvants that are utilized to stimulate anticancer immune responses, ligands of the stimulator of interferon genes (STING) pathway have received increasing attention. STING activation can trigger dendritic cell (DC) activation and endogenous immune responses, thereby preventing tumor escape. Activation of the STING pathway in the context of ACT was accordingly associated with improved T cell trafficking and persistence in the TME combined with the reduced presence of immunosuppressive cells. Recent findings also suggest cell-intrinsic effects of STING ligands on T cells. Activation of the STING signaling pathway was in this regard shown to enhance effector functions of CD4+ and CD8+ T cells, suggesting that the STING signaling could be exploited to harness T cell anticancer functions. In this review, we will discuss how the STING signaling can be used to enhance the anticancer efficacy of ACT.

Keywords: Adoptive T cell therapy, STING, T cells, cancer, immunomodulation.

Abbreviations:

THE RELEVANCE OF ADOPTIVE T CELL THERAPY IN CANCER
In 2018, Tasuku Honjo and James Allison were awarded the Nobel Prize for their research on immune checkpoint inhibitors (ICIs). They found that inhibition of the suppressive molecules PD-1 and CTLA-4 enhanced the ability of the immune system to eliminate cancer cells [1]. Immune checkpoints are expressed on activated immune cells and serve to maintain self-tolerance and regulate immune responses. Tumor-induced engagement of these immune checkpoints is considered as a resistance mechanism that limits T cell activation [2]. Numerous anti-PD-1 and anti-CTLA-4 therapies were developed and showed unparalleled survival rates for patients with non-small cell lung cancer, renal cell carcinoma, and melanoma [3]. Although these treatments are now FDA-approved for many cancer types, a significant number of patients suffers from immune-related adverse effects or acquired resistance [4]. Especially in weakly immunogenic cancers, some patients cannot benefit from ICIs, highlighting the importance of further progress in this research area and the need for alternative therapeutic approaches [5]. One other treatment avenue with proven clinical success is adoptive cell therapy (ACT). In ACT, autologous immune cells are amplified ex-vivo, modified, and subsequently transferred back into the patient [6]. It has been successfully demonstrated that ICI and
ACT can be combined [7, 8], possibly providing additional benefits [9]. Some metastatic melanoma patients in whom ICI showed no therapeutic effect were successfully treated with ACT, suggesting that ACT can be used when ICIs fail [10-13].

Three main T cell-based approaches are currently considered for ACT, including the use of tumor-infiltrating lymphocytes (TILs), antigen-specific T cells equipped with a specific T cell receptor (TCR), and chimeric antigen receptor T cells (CAR-T) [14]. TCR-based ACT does not require an engineered construct but relies on the target antigen being presented via the major histocompatibility complex (MHC). CAR-T based ACT can target MHC independent antigens, as well as carbohydrates and glycolipids expressed on the cell surface of tumors [14]. CD8+ T cells can directly eliminate tumor cells due to their cytotoxic properties, which is why they have long been considered as the most suitable T cell subset for ACT. CD8+ T cell-mediated killing occurs through MHC-i-dependent recognition of tumor cells and subsequent granzyme- and/or FAS ligand-dependent elimination. This also requires cross-priming by DCs as well as co-stimulation by natural killer cells and/or CD4+ T cells derived cytokines [15]. However, the support of innate immunity to T cell adaptive immune responses is not always present in the TME. If CD8+ T cells cannot be primed and activated in the TME, CD8+ T cell-dependent tumor elimination fails [16]. The required co-stimulation to activate CD8+ T cells can also be provided by CD4+ T cells. Current strategies aim to exploit these properties of CD4+ T cells in ACT [17]. CD4+ T cells are also suitable for ACT because they support antigen presentation from DCs, T cell homing through the secretion of chemokines such as CXCL9-11, formation of CD8+ T cell memory, and direct tumor elimination by granymes, perforin, TRAIL, or Fasl (reviewed in [18]). A recent study examining long-lasting anti-CI9 CAR T cells demonstrated that 9 years after therapy, the long-term protection against CD19+ cells was mediated almost exclusively by cytotoxic CD4+ CAR T cells. This suggests that beyond the short-term tumor elimination mediated by CD8+ T cells, CD4+ T cells contribute to long-term remission [19].

Because it is now accepted that the immune system shapes the initiation and progression of cancer [20], increasing efforts are being made to design and exploit adjuvants that will boost anticancer immune responses. Additional adjuvants include agonists of pattern recognition receptors, such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs) and cytosolic DNA sensors such as stimulator of interferon genes (STING) [21].

Two major uses for STING agonist-mediated immune cell infiltration, combination therapies with ICIs were tested [28]. Promising preclinical results when combining ICIs and STING agonists were achieved in multiple cancer models, including melanoma and an HPV+ oral tumor [29-32]. However, these successes failed to translate into the clinic due to the pharmacokinetics of STING agonists that preclude effective drug delivery [33]. Furthermore, some tumors feature limited responses to STING ligands due to inhibitory mechanisms that include for instance p53 [34], ecto-nucleotide pyrophosphatases 1 (ENPP1) [35], Hypoxia-induced RNASEH2a upregulation [36], and TIM-3 [37]. To tackle some of these hurdles, a better bioavailability of STING agonists is needed. This could be achieved through more stable STING agonists or improved delivery systems (discussed in [33]).

STING-DRIVEN ACTIVATION OF INNATE IMMUNE CELLS FAVORS T CELL ACTIVATION

Two major uses for STING activation can be contemplated. The first is to deliver STING agonists directly into the TME of the host. This approach can trigger anticancer responses [38], thereby contributing to overall tumor elimination as discussed above. Macrophages and DCs are critical innate immune cells that affect CD8+ T cell-mediated tumor elimination. Type-2 macrophages can be repolarized into type-1 macrophages by STING activation, which can improve the antitumor response by enhancing the co-stimulation and differentiation of CD4+ and CD8+ T cells [39]. In DCs, cGAS-STING is required for antigen presentation and cross-priming of T cells [27]. DC-mediated cross-priming is fol-
lowed by recruitment of cytotoxic T cells to the TME through the chemokines CXCL9 and CXCL10 [37, 40].

Numerous studies (discussed in [24]) showed that disruption of the STING-axis led to compromised CD8+ T cell-mediated tumor elimination. STING activation in the TME therefore supports T cell functions. This was exploited using either CD8+ and CD4+ CAR T cells, as discussed further below.

Using a murine second-generation specific CD8+ CAR T cell model, the group of Sandra Hervas-Stubbs demonstrated that 2’3’-cGAMP treatment induced an endogenous T cell response, prevented antigen-loss variant outgrowth and led to an improved overall survival rate [42]. By using a bilateral B16-OVA tumor mouse model, the authors observed restrained tumor progression in the injected and opposite tumor, when the combination of 2’3’-cGAMP injection and antigen-specific CD8+ CAR-T cells was used. Most importantly, mice treated with the combination were the only ones to survive, while the control groups receiving
Jonathan Serody’s group recently used mouse CD4+ T helper cells in combination with STING agonist to treat a locally advanced breast cancer model [43]. While the potential of CD4+ T cells for ACT is clear, the diversity of CD4+ T cell responses should be considered. CD4+ T cells are a highly heterogeneous group consisting of T\(_{h}1\), T\(_{h}2\), T\(_{h}9\), T\(_{h}17\), T\(_{reg}\), and T\(_{m}1\) cell subsets. Each is characterized by a subset-specific cytokine profile and thereby fulfills different effector functions [44]. Although there is a large agreement that Tregs and T\(_{h}1\) cells respectively favor and restrict tumor progression, T\(_{h}2\), T\(_{h}9\) and T\(_{h}17\) cell functions in cancer are context-dependent [45]. T\(_{h}17\) cells can contrastingly affect cancer depending on their environment [46]. However, in the setting of adoptive cell therapy, IL-17-secreting T cells are considered beneficial, with reported antitumor activity in melanoma and lung cancer [47-49].

The effect of DMXAA and 2’3’-cGAMP in combination with murine Neu-specific T\(_{h}1/T_{h}17\) CAR T cells was investigated in the setting of breast cancer [43]. The authors have demonstrated that the combined treatment mediates antitumor control due to improved trafficking and persistence of CAR T cells in the TME [43]. T\(_{h}1/T_{h}17\) CAR T cells featured improved antitumor efficacy in mice receiving DMXAA, resulting in long-term control in some of the treated mice, while standalone treatments remained ineffective [43]. The infiltrating CAR-T cells were then analyzed by flow cytometry to determine the cause of the enhanced antitumor response. The authors found that some of the transferred T\(_{h}1/T_{h}17\) CAR-T cells had undergone a shift to T\(_{h}1\) cells and that DMXAA enhanced the accumulation of these T\(_{h}1/T_{h}17\) CAR-T cells in the TME. This phenotypic shift was accompanied by the upregulation of T\(_{h}1/T_{h}17\) signature transcription factors and cytokines (7bx21 and IFN-γ). By using anti-IFN-γ mAb, they found that tumor elimination occurred in a T\(_{h}1/T_{h}17\) CAR-T cell mediated manner [43]. It was previously shown that T\(_{h}17\) cells possess some levels of plasticity enabling their transformation into IFN-γ-producing T\(_{h}1\)-like cells [50]. This plasticity is necessary for their antitumor response in T\(_{h}17\) ACT [51]. However, the observation that activation of the STING signaling pathway further enhances this plasticity and the antitumor responses is of high interest for cancer immunotherapy. Despite the improved effect of the combined CAR T cell and STING agonist treatment, one question remains: Why do most tumors recur as IL-17-secreting T cells are known to be long-lived and self-renewing with superior persistence [52]? To address this, the authors have performed a single-cell transcriptome sequencing of CD45+ cells from the TME. The authors compared leukocytes on the day of best tumor control with their counterparts collected on the day of tumor recurrence. In DMXAA treated mice, they found a shift in the myeloid cells of the TME favoring M1-like macrophages. Interestingly, their depletion by means of liposome clodronate was associated with a complete loss of the DMXAA-induced therapeutic benefit. This shift was accompanied by a stronger M1 gene expression including nos2 and inhba, and a reduced expression of M2 associated genes like retnla, mrc1, fak2, and il10. Furthermore, M1-associated chemokines (cxcl9, cxcl10, and ccl5) were
increasingly secreted to attract T<sub>H</sub>1/T<sub>H</sub>17 T cells to the TME. In addition, they have found that when the combined therapy transiently relieves immunosuppression in the TME, this effect is short-lived and immunosuppressive myeloid cells were subsequently present in the TME. They also analyzed the T<sub>C</sub>/T<sub>C</sub>17 CAR T cells from the TME and discovered an increased expression of markers associated with dysfunction and apoptosis, providing evidence for their assumption that T cell dysfunction is the key limiting factor of the therapy. This suggested that both immunosuppression driven by myeloid cells and T cell dysfunction were responsible for the absence of complete responses to the combined therapy. To test this further, the combination of T<sub>C</sub>/T<sub>C</sub>17 CAR T cells and DMXAA was administered with anti-PD-1 and anti-GR-1 monoclonal antibodies twice a week, starting one day after the CAR T cell injection. This resulted in a marked increase in the ability of tumor-bearing mice to reject tumors. Importantly, only the combination of both antibodies was effective, indicating that both PD-1/L and myeloid cell-driven immunosuppression needed to be targeted to yield a therapeutic benefit. Next, the authors examined the effect of their T<sub>C</sub>/T<sub>C</sub>17 CAR T cells by comparing them with CAR T cells expanded with IL-7 and IL-15. The use of T<sub>C</sub>/T<sub>C</sub>17 CAR T cells resulted in an improved in vivo antitumor efficacy over the latter [43]. The authors linked this difference to the significantly higher proliferation rate of CD<sup>4</sup>+ CAR T cells in the spleen and the enhanced expansion of CD<sup>8</sup> CAR T cells with a central memory phenotype. Finally, the authors compared DMXAA with the other STING agonist 23'-cGAMP. Although the use of 23'-cGAMP resulted in significantly more T<sub>C</sub>/T<sub>C</sub>17 CAR T cells in the TME, there was only a minor difference in therapeutic efficacy [43]. Overall, this study suggests that STING ligands and CAR T cells have synergistic effects that can successfully fight breast cancer when combined.

**T CELL-INTRINSIC STING ACTIVATION AND ITS RELEVANCE IN ACT**

Direct administration of STING agonists faces obstacles such as difficulties with drug delivery and poor pharmacokinetics. It is further accompanied by reports of autoimmune events after excessive STING stimulation [33]. To circumvent this, cells can be directly activated with the STING agonist prior to transfer. This would prevent a STING-mediated detrimental inflammatory response in the host and minimize drug-induced adverse events. We will therefore next focus on the intrinsic effect of STING agonists in T cells and discuss how STING signaling affects their viability, cell proliferation, cytokine secretion, and antitumor functions (Figure 2).

Alexander Poltorak’s team studies on mouse T cells showed that the STING signaling pathway, already shown to be active in macrophages, was also functional in T cells. In T cells, STING induction with DMXAA leads to IFN-β production, prevention of cell proliferation, and induction of proapoptotic genes. However, these cell-adverse effects were not observable with low doses of DMXAA [53]. Andrea Ablasser’s group confirmed the cell death-inducing effect of the STING agonists CMA and DMXAA, but additionally found that this was due to an intensified STING signaling response in T cells as compared to macrophages and DCs [53, 54]. It should be noted that those findings were mostly established using synthetic STING ligands such as CMA and DMXAA. By contrast, other studies reported limited apoptosis induction upon treatment of T cells with 23'-cGAMP [42, 55]. Liufu Deng’s laboratory instead investigated whether STING signaling in CD8<sup>+</sup> T cells was necessary for their antitumor effects. They found that the cGAS-STING pathway was required in CD8<sup>+</sup> T cells to induce an antitumor response [56]. The use of murine tumor-specific T cells lacking either cGAS or STING showed reduced antitumor activity as compared to controls. Likewise, conditional knockout of STING in CD4<sup>+</sup> T cells and STING<sup>−/−</sup> mice resulted in accelerated tumor progression in the mouse T cell lymphoma model EG7 and mouse glioblastoma model GL261. Examination of proliferative capacity and effector functions revealed that cGAS- and STING-deficient CD8<sup>+</sup> T cells displayed impaired proliferation rate after adoptive transfer as compared to controls. In addition, the number of TNF-α<sup>+</sup> and IFN-γ<sup>+</sup> CD8<sup>+</sup> T cells in tumor draining-lymph nodes was significantly reduced, suggesting dysfunctional effector functions. Finally, the authors showed that cGAS or STING deficiency in CD8<sup>+</sup> T cells led to an accumulation of effector CD8<sup>+</sup> T cells at the expense of self-renewing and persistent central memory CD8<sup>+</sup> T cells. cGAS- or STING-deficient CD8<sup>+</sup> T cells also exhibited a terminally exhausted phenotype.

Collectively, the authors’ findings support the fundamental role of the cGAS-STING axis in CD8<sup>+</sup> T cells for ACT [56]. Importantly, in line with the observations of other investigators [42, 55], STING treatment did not affect the cell viability of the human CD8<sup>+</sup> T cells used [56]. In summary, an intact cGAS-STING axis in CD8<sup>+</sup> T cells is required for their anticancer effector functions. Because excessive STING activation in T cells may trigger cell death, it remains important to carefully consider the dose of the STING ligands used to harness T cell effector functions without compromising their proliferation or viability. We have accordingly shown that STING ligands can enhance T cell effector functions and the differentiation of T<sub>H</sub>1 and T<sub>H</sub>9 cells [55].

T<sub>H</sub>9 cells are defined as cells lacking Foxp3 but secreting high levels of IL-9 [57]. They also secrete IL-10 and IL-21 [58, 59]. Polarization of naive T cells into T<sub>H</sub>9 cells is initiated by IL-4 and TGF-β and requires a complex interaction of a network of transcription factors, including IRF4, GATA3, BATF3, and PU.1 [60]. T<sub>H</sub>9 cells are particularly promising in the context of ACT, as Puwar and colleagues demonstrated superior antitumor properties of T<sub>H</sub>9 cells upon adoptive transfer as compared to other CD4<sup>+</sup> subsets in a mouse model of melanoma [61]. Their superiority was subsequently independently demonstrated by multiple laboratories [58, 62, 63]. Adoptively transferred tumor Ag-specific murine T<sub>H</sub>9 cells were shown to provide superior antitumor immunity by eliminating variants with antigen loss [64]. All these findings provide impetus to investigating the relevance of T<sub>H</sub>9 cells in the ACT of cancer.
Investigations conducted by our group using different subsets of CD4+ T cells, including Th1, Th9, and Th17 cells, which were directly activated with different STING ligands, revealed that the differentiation and effector functions of Th1 and Th9 cells could be enhanced by STING activation [55].

We further demonstrated that Th1 and Th9 cells respond differently to STING ligands, as illustrated by our observation that Th1 cells were more sensitive to STING ligand-induced apoptosis than Th9 cells. STING activation enhanced human Th1 and Th9 polarization, and resulted in increased expression and secretion of IFN-γ and IL-9, the respective Th1 and Th9 signature cytokines [55]. These results showing that ligands of STING enhance Th9 cell differentiation are in line with published investigations indicating that pro-inflammatory components such as glucocorticoid-induced TNFR-related protein (GITR), IL-1β, TNFα, OX40L, and TL1A support Th9 effector functions [58, 65-68].

It is noteworthy that distinct mechanisms were contributing to the cell-intrinsic STING-driven enhancement of Th1 and Th9 differentiation. IRF3 activation was essential for the STING-mediated induction of Th1 cell differentiation, while mTOR signaling accounts for the increased Th9 cell differentiation following STING activation [55]. We tested the in vivo functions of Th9 cells treated with 2’3’-cGAMP in the B16-OVA melanoma model. For both subcutaneously or intravenously injected B16-OVA cells, we demonstrated that adoptively transferred 2’3’-cGAMP-stimulated tumor Ag-specific Th9 cells secreted more IL-9 and triggered better antitumor immunity compared to controls without STING agonists [55]. These results show that STING activation can enhance the anticancer efficacy of adoptively transferred T cells (Figure 2).
CONCLUSIONS

While STING function was initially characterized in fibroblasts [69], it is now clear that STING shapes the biology of multiple immune cell types, including T cells. STING agonists have the potential to address some of the critical challenges of adoptive cell therapy. STING agonists can indeed enhance T cell infiltration and reduce tumor-induced immunosuppression [42, 43]. Despite these promising advances, the disappointing results obtained when combining STING agonists with ICIs underscore the challenge to translate the use of STING agonists into the clinic. Documented issues such as toxicity, low bioavailability, and related difficulties of administration likely prevent the clinical implementation of STING ligands against cancer (discussed in [70]). In that regard, the recent work of Jneid et al. that relies on the use of virus-like particles to deliver cGAMP in the TME highlights an elegant venue to circumvent some of these issues [71]. Recent results suggest that direct T cell activation by STING agonists can be exploited in the context of ACT. Both Liu Fu Deng’s laboratory and ours have shown that T cells can be directly activated with STING agonists without triggering marked cell death [55, 56]. A thoughtful selection and careful use of STING ligands will allow harnessing of T cell anticancer functions without compromising their fitness. Further research is warranted to translate the therapeutic use of STING ligands in the setting of ACT.

REFERENCES


STING-driven activation of T cells in ACT of cancer


