Dynamic analyses of tumor microenvironment modulation by CAN-2409 treatment using Kaede photoconvertible transgenic mice

David R. Withers[1], Anne R. Diers[2], Zhi Li[1], Suaad Idris[1], Claire Willis[1], Paul Peter Tak[2], Francesca Barone[2]

[1]University of Birmingham Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, Birmingham, UK; [2]Candel Therapeutics, Inc., Needham, MA, USA

Abstract Number: 1134

Background

- CAN-2409 is a replication-defective adenovirus that delivers the HSV-thymidine kinase gene.
- Intratumoral administration of CAN-2409 followed by prodrug results in the formation of a toxic metabolite able to induce immunogenic cell death, exposure of tumor-associated antigens, and activation of local and systemic immune responses against the tumor.
- Biomarker analysis from ongoing clinical trials demonstrated systemic activation of lymphocytes, increased infiltration of cytotoxic T cells and increased T cell aggregation in proximity to tumor cells in tumor biopsies after treatment with CAN-2409 [1].
- Use of state-of-the-art preclinical tools for dynamic assessment of the lymphocyte response in vivo will enable assessment of the evolution of the anti-tumor immune response induced by CAN-2409 and provide the immunological rationale for potential therapeutic combinatory approaches.

CAN-2409 mechanism of action

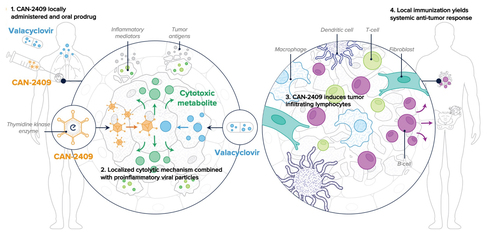
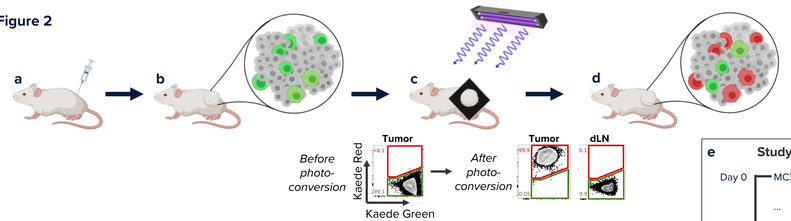


Figure 1. Administration of CAN-2409 plus prodrug results in local and systemic activation of the immune system against newly released tumor antigens.

Methods

Assessment of anti-tumor immune responses using Kaede photoconvertible transgenic mice

Figure 2



Murine colon adenocarcinoma (MC38) cells are implanted subcutaneously into photoconvertible C57BL/6 Kaede mice (Fig. 2a-b) . Violet light is used to simultaneously label the entire tumor microenvironment (TME, Fig. 2c), enabling the discrimination of **cells retained within the tumor** (**Kaede Red+**) versus **newly entering cells** (**Kaede Green+**, Fig. 2d) and the ability to assess real-time changes occurring in the immune compartment of the TME [2]. Scheme created with BioRender.com.

In the studies presented, CAN-2409 is administered intratumorally (i.t., $3x10^{10}$ viral particles in 20uL) on day 10 after tumor inoculation. CAN-2409 administration is followed by prodrug (ganciclovir, 50mg/kg IP QD on day 11 up to 14, Fig. 2e).

e Study Timeline

Day 0	MC38 tumor inoculation
...	...
Day 10	Sham or CAN-2409 i.t.
Day 11	Prodrug
Day 12	Prodrug Tumor photoconversion
Day 13	Prodrug
Day 14	Tumor collection

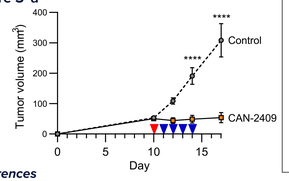
N=6 mice per group

Anti-tumor activity of CAN-2409

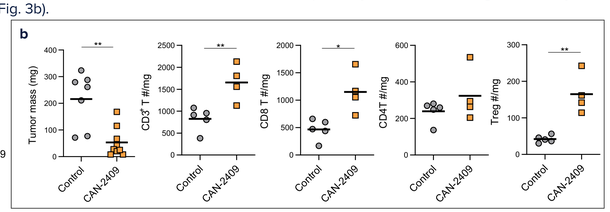
CAN-2409 inhibits tumor growth and increases lymphocyte infiltration in MC38 tumor-bearing C57BL/6 mice

MC38 tumor-bearing mice were treated with or without CAN-2409 i.t. (red arrowhead) followed by 4 days of i.p. administration of prodrug (blue arrowheads). Significant tumor growth inhibition was observed with CAN-2409 treatment (Fig. 3a). Endpoint analysis of tumor immune populations assessed by flow cytometry demonstrated an increase in CD3+ cells, due primarily to CD8 T and Treg cells. 2-way ANOVA, * p < 0.05, ** p < 0.01, **** p < 0.0001 (Fig. 3b).

Figure 3 a



b



References

[1] Abstract #9037, 2022 ASCO Annual Meeting

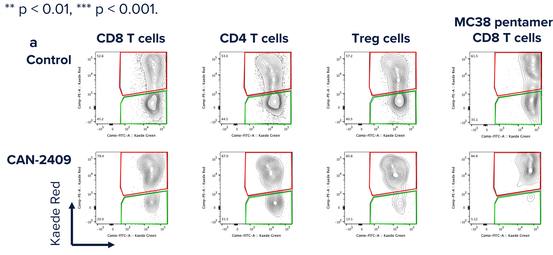
[2] J Exp Med (2022) 219(6):e20210749. https://doi.org/10.1084/jem.20210749

Dynamic analyses of tumor microenvironment (TME) modulation by CAN-2409

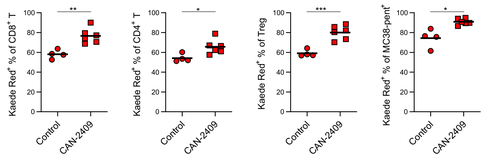
CAN-2409 induces expansion of retained immune cells in tumors

Representative flow cytometry plots showing Kaede Red and Kaede Green expression in immune subpopulations within tumors. MC38 pentamers were used to identify antigen specific CD8 T cells. Quantification of retained (KR+) cells demonstrates an increase in each T cell subset after CAN-2409 treatment. Fig. 4a representative dot plots and b. quantification of all animals, n=4-6, unpaired t test, * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 4 a



b

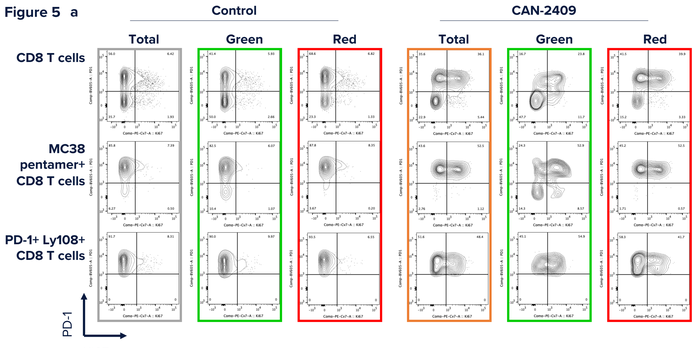


CAN-2409 stimulates proliferation of recruited and retained T cell subsets in the TME

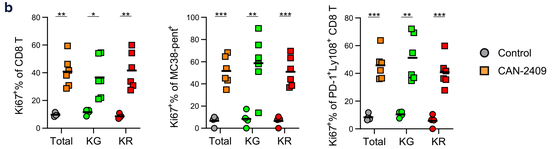
Ki67 staining demonstrated significant increase in T cell proliferation upon CAN-2409 treatment in both the newly recruited and retained compartments for total and antigen-specific (MC38 pentamer+) CD8 T cells.

Further analysis of the PD-1+ Ly108+ CD8 T cells confirmed significant proliferation of both effector and stem-like CD8 T cells in both newly recruited and retained T cells. Fig. 5a representative dot plots and 5b quantification of all animals, n=4-6, unpaired t test, * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 5 a



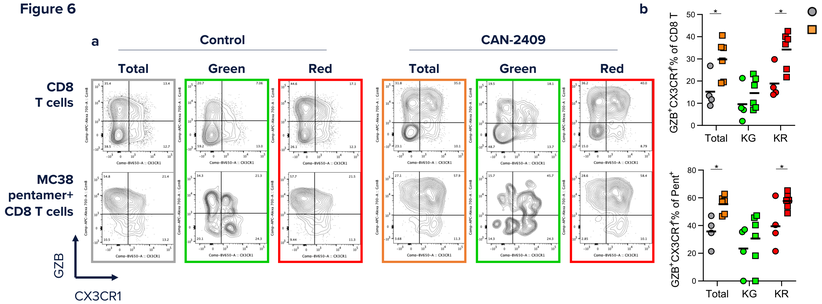
b



CAN-2409 reinvigorates tumor-retained, exhausted neoantigen-specific CD8 T cells

Treatment with CAN-2409 induced a striking increase of GZB+ CX3CR1+ CD8 T cells retained in the tumor (KR), suggesting the ability of CAN-2409 to reinvigorate exhausted (CX3CR1 negative) CD8 T cells. Fig. 6a illustrates representative flow cytometry plots for total, Kaede Green+ (KG), and Kaede Red+ (KR) populations of total and antigen-specific (MC38 pentamer+) CD8 T. Quantification of changes in all groups, n=4-6, is represented in Fig. 6b, unpaired t test, * p < 0.05.

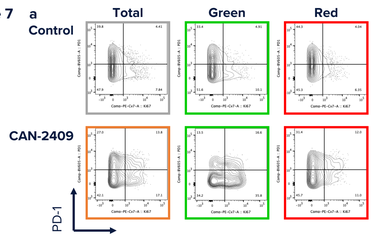
Figure 6



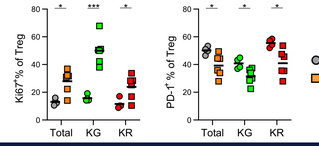
Newly recruited and retained Treg cells adopt an altered phenotype in CAN-2409 treated tumors

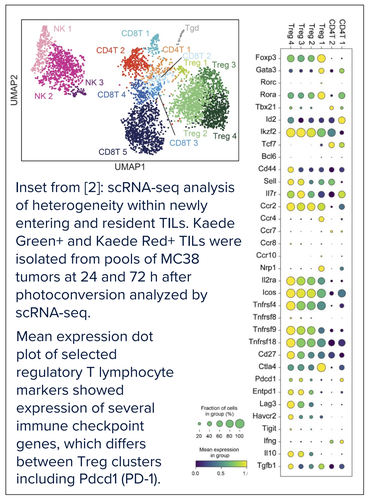
Upon treatment with CAN-2409, increased proliferation of a Treg population characterized by decreased PD-1 expression was observed. Changes are suggestive of a decreased functional state of this population (see inset). Representative flow cytometry plots for total, Kaede Green+ (KG), and Kaede Red+ (KR) Treg cells are shown (Fig. 7a). Quantification for all treated animals, n=4-6 (Fig. 7b), unpaired t test, * p < 0.05, *** p < 0.001.

Figure 7 a



b





Inset from [2]: scRNA-seq analysis of heterogeneity within newly entering and resident TILs. Kaede Green+ and Kaede Red+ TILs were isolated from pools of MC38 tumors at 24 and 72 h after photoconversion analyzed by scRNA-seq.

Mean expression dot plot of selected regulatory T lymphocyte markers showed expression of several immune checkpoint genes, which differs between Treg clusters including Pdcd1 (PD-1).

Conclusions

- CAN-2409 alters the TME such that newly entering and retained CD8 T cells expand and maintain key effector functions while the exhausted CD8 compartment is reinvigorated.
- Newly recruited and retained Treg cells also adopt an altered phenotype, suggestive of a decreased functional state, in CAN-2409 treated tumors.
- Collectively, these data suggest at least two temporally distinct pathways underpinning CAN-2409 systemic mode of action – CAN-2409 treatment overcomes cell exhaustion and decreases immune suppression – supporting the rationale for use of CAN-2409 either as monotherapy or in combination.

Presented at the 2022 Society for Immunotherapy of Cancer (SITC) Annual Meeting

Contact email: info@candeltx.com