

1134

## DYNAMIC ANALYSES OF TUMOR MICROENVIRONMENT MODULATION BY CAN-2409 TREATMENT USING KAEDE PHOTOCONVERTIBLE TRANSGENIC MICE

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.1134>

<sup>1</sup>David Withers\*, <sup>2</sup>Anne Diers, <sup>1</sup>Zhi Li, <sup>1</sup>Suaad Idris, <sup>1</sup>Claire Willis, <sup>2</sup>Paul Peter Tak, <sup>2</sup>Francesca Barone. <sup>1</sup>University of Birmingham, Birmingham, UK; <sup>2</sup>Candel Therapeutics, Needham, MA, USA

**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. 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.

**Methods** We utilized a dynamic labeling model<sup>1</sup> where MC38 tumor cells were implanted subcutaneously into photoconvertible Kaede mice; violet light was used to simultaneously label the entire tumor microenvironment (TME), enabling the discrimination of cells retained within the tumor (Kaede Red+) versus newly entering cells (Kaede Green+) and the ability to assess real-time changes occurring in the immune compartment of the TME. CAN-2409 was administered intratumorally on day 10 after tumor inoculation followed by prodrug (ganciclovir, 50mg/kg IP QD on days 11 to 14). Photoconversion occurred on day 12, and analysis was performed 48h later. Dynamic of MC38-tumor specific T cell clones was evaluated by flow cytometry. We also explored the effect of CAN-2409 in combination with immune checkpoint inhibition (ICI).

**Results** Administration of CAN-2409 led to control of tumor growth and a significantly increased effector CD8 T cell response. Photolabeling of the TME revealed that rather than enhancing recruitment of T cells to the tumor, CAN-2409 altered the TME such that newly entering, but also retained CD8 T cell were significantly more proliferative. Amongst the retained population, terminally exhausted neoantigen-specific CD8 T cells showed evidence of reinvigoration, adopting a CX3CR1+ GZMB+ phenotype. There was also enhanced proliferation within the PD-1+ stem-like compartment of newly recruited cells and we observed expansion of a population of non-activated, likely less suppressive, Tregs. The combination of CAN-2409 and anti-CTLA-4 (clone 9H10) treatment further improved control of tumor growth and remodeled the Treg compartment to further skew the Treg:CD8 ratio in favor of the effector response.

**Conclusions** CAN-2409 alters the TME such that newly entering CD8 T cells expand and retain key effector functions while the exhausted CD8 compartment is reinvigorated, likely reducing Treg-mediated suppression. Collectively, these data suggest at least two temporally distinct pathways underpinning CAN-2409 action that overcome cell exhaustion and decreased immune suppression, supporting the rationale for the use of CAN-2409 either as monotherapy or in combination.

### REFERENCE

1. Zhi Li, Zewen K Tuong, Isaac Dean *et al.* *In vivo* labeling reveals continuous trafficking of TCF-1+ T cells between tumor and lymphoid tissue. *J Exp Med* 2022;**219**(6):e20210749. doi: 10.1084/jem.20210749

**Ethics Approval** All *in vivo* experiments were conducted in accordance to UK HO guidelines on an approved PPL