# Therapeutic potential of CAN-3110 in Ras-Raf pathway altered melanoma

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## CAN-3110 mechanism of action

- CAN-3110 is a first-in-class, replication-competent herpes simplex virus-1 (HSV-1) oncolytic viral immunotherapy candidate designed with dual activity for oncolysis and immune activation under development in recurrent high-grade glioma.
- CAN-3110 is engineered with deletion of the viral ICP6 gene and expression of the viral replication factor ICP34.5 under the control of a mammalian nestin-hsp68 transcriptional promoter/enhancer cassette.
- These modifications confer selective replication in tumors with nestin expression [1-3] and CDKN2A loss-of-function [4].

**Figure 1**

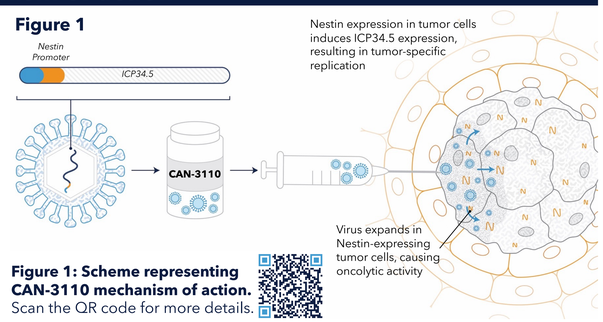


**Figure 1: Scheme representing CAN-3110 mechanism of action.** Scan the QR code for more details.

## High nestin expression and CDKN2A loss-of-function: features of skin cutaneous melanoma

- Melanoma is characterized by high nestin expression (Fig. 2).
- Frequent CDKN2A loss-of-function and additional alterations in the Ras-Raf pathway are observed in melanoma samples. Figure 3 illustrates mutation profile of melanoma as compared to glioblastoma multiforme.
- Taken together, these data provide the rationale for development of CAN-3110 in this tumor type.
- **Here, we assessed the anti-tumor activity and mechanism of action of CAN-3110 in preclinical melanoma models to support development in this indication.**

**Figure 2**

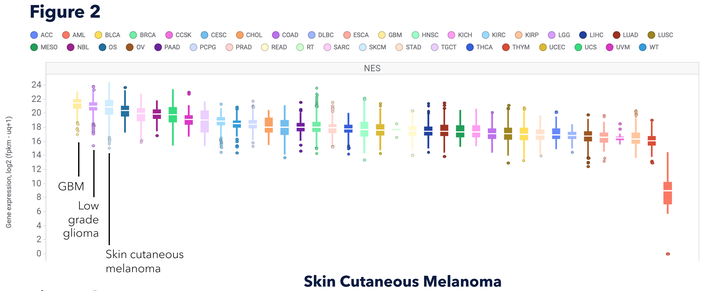


**Figure 2:** RNASeq data from NCI Genomic Data Commons normalized using the USCS Xena webtool. Nestin (NES) expression for 10,890 solid tumors is shown. Glioblastoma multiforme (GBM), brain low grade glioma (LGG), skin cutaneous melanoma (SKCM).

**Figure 3**

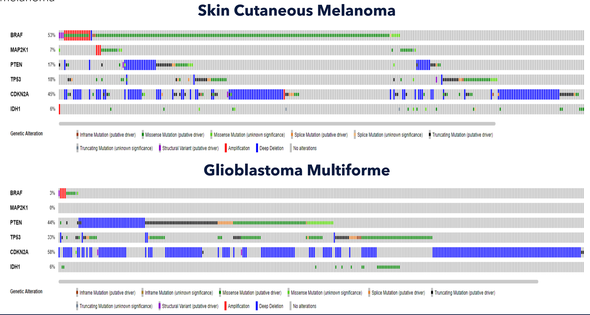


**Figure 3:** An OncoPrint visualization comparing genetic and genomic alterations prevalent in skin cutaneous melanoma (N=363) and glioblastoma multiforme (N=378) tumors (cBioPortal, accessed May2024). Data from TCGA, PanCancer Atlas.

## CAN-3110 induces viral replication-mediated cytotoxicity in human melanoma cell lines

We tested in vitro cytotoxicity of a GFP-expressing analog of CAN-3110 [2] in two melanoma cell lines (Hs-695T and SK-MEL-28) and human dermal fibroblasts (HDF, Table 1). Both melanoma cell lines were permissive to infection (Fig. 4) and displayed similar cytotoxicity profiles after infection, distinct from HDF (Fig. 5, 6), thus confirming tumor-specific cytotoxicity of CAN-3110.

**Table 1**

| Identifier | Cell type | Transformed | Nestin expression | CDKN2A | CDK4 | BRAF |
|---|---|---|---|---|---|---|
| HDF | Human dermal fibroblasts | No | Low | WT | WT | WT |
| Hs-695T | Human melanoma | Yes | High | WT | WT | Mut (V600E) |
| SK-MEL-28 | Human melanoma | Yes | Low | WT | Mut* (R24C) | Mut (V600E) |

*Abolishes the ability of CDK4 to bind to $p16^{INK4A}$ resulting in CDKN2A pathway dysfunction

**Figure 5**

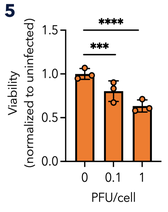


**Figure 5:** HDF were infected with an analog of CAN-3110 expressing GFP. Cell viability was tested 5 days after infection. N=3, ***p<0.001, ****p<0.0001.

**Figure 6**

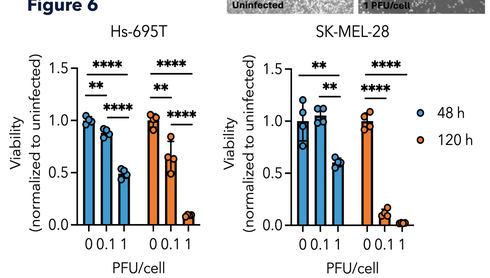


**Figure 6:** Hs-695T and SK-MEL-28 cell lines were infected with a GFP-expressing analog of CAN-3110. Cell viability was tested at 2 and 5 days after infection. N=4, **p<0.01, ****p<0.0001.

**Figure 4**




**Figure 4: A.** Representative images of Hs-695T and SK-MEL-28 5 days post-infection with 1 PFU/cell. 10X magnification. **B.** GFP-positive cells were assessed by flow cytometry. N=4, *p<0.05, **p<0.01, ***p<0.001.

## Development of an engineered murine melanoma model for assessment of CAN-3110

The use of a nestin-expressing, syngeneic melanoma allograft model would be ideal for assessment of CAN-3110 anti-tumor activity and associated immune response; however, B16-F10 cells are not permissive to HSV-1 infection [5]. Thus, B16-F10 murine melanoma cells were engineered to express a human receptor for HSV-1 infection, Nectin1 (termed B16-F10-Nectin1-hi). This modification did not alter nestin expression nor affect tumor growth rate in vivo (Fig. 8). B16-F10-Nectin1-hi cells were likewise permissive to infection with a GFP-expressing CAN-3110 analog (Fig. 9) and supported viral replication-mediated cytotoxicity in vitro (Fig. 10).

**Figure 7**

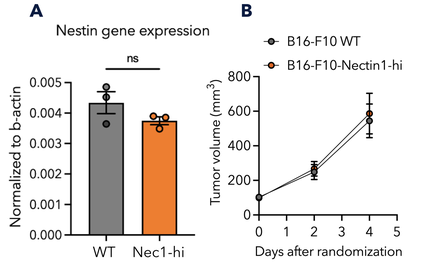


**Figure 7: A.** Gene expression analysis of the wild-type (WT) B16-F10 (WT) and engineered B16-F10-Nectin1-hi (Nec1-hi) cells for nestin. N=3, NS: not significant. **B.** Tumor growth curves for WT and B16-F10-Nectin1-hi cells implanted subcutaneously in C57BL/6 mice. N=4.

**Figure 8**

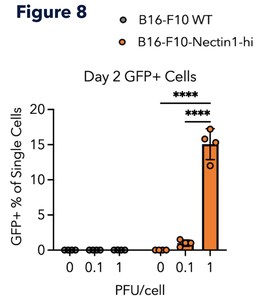


**Figure 8:** GFP-positive cells were assessed by flow cytometry after infection with a GFP-expressing analog of CAN-3110 in wild-type (WT) B16-F10 and B16-F10-Nectin1-hi cells. N=4, ****p<0.0001.

**Figure 9**

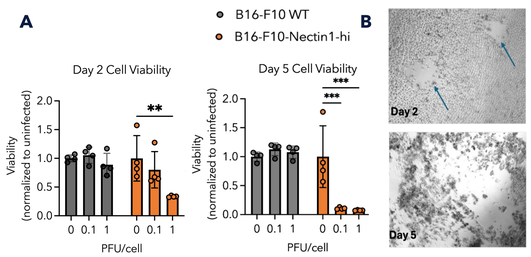


**Figure 9: A.** Cell viability was assessed by RealTime-Glo after infection with a GFP-expressing analog of CAN-3110 in wild-type (WT) B16-F10 and B16-F10-Nectin1-hi cells. N=4, **p<0.01, ***p<0.001. **B.** Representative images of B16-F10-Nectin1-hi cells 2- and 5-days post-infection with 0.1 PFU/cell of a GFP-expressing analog of CAN-3110. Arrows: cytopathic plaques. 10X magnification.

## CAN-3110 displays in vivo anti-tumor activity in an engineered murine melanoma model

In vivo, intratumoral treatment with CAN-3110 resulted in dose-dependent inhibition of tumor growth compared to the vehicle control group, and three of 8 tumors regressed in the dose [high] group (Fig. 11). Treatment with CAN-3110 was well-tolerated based on organ and body weight (Table 2, Fig. 12). Further, anti-tumor activity was associated with systemic immune activation including activation of CD8+ and CD4+ T cells, NK cells, and gamma/delta T cells (Fig. 13).

**Figure 10**

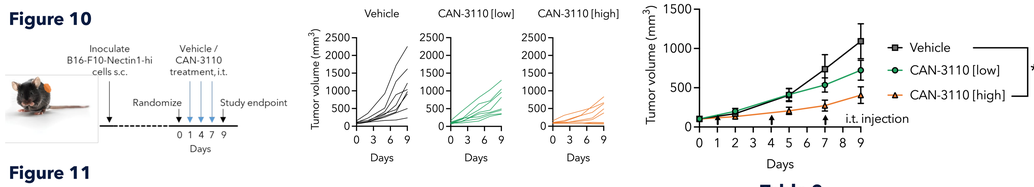


**Figure 10:** In vivo study design and tumor growth over time after treatment with CAN-3110 ([low] 2.5E5 pfu per injection, [high] 2.5E6 pfu per infection). N=8 per group. *p<0.05.

**Figure 11**

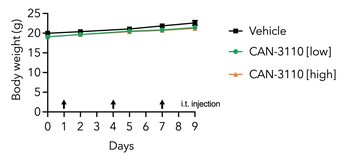


**Figure 11:** Body weights of B16-F10-Nectin1-hi tumor-bearing mice after CAN-3110 treatment. No statistically significant changes in body weight were found.

**Table 2**

| | Tissue weight at endpoint (g) | | | |
|---|---|---|---|---|
| | Vehicle | CAN-3110 [low] | CAN-3110 [high] | Statistic |
| Tumor | 2.386 ± 1.711 | 1.444 ± 0.977 | 0.836 ± 0.763 | p = 0.0479* |
| Liver | 1.017 ± 0.091 | 1.058 ± 0.174 | 1.093 ± 0.158 | NS |
| Heart | 0.115 ± 0.017 | 0.106 ± 0.007 | 0.104 ± 0.012 | NS |
| Spleen | 0.196 ± 0.061 | 0.162 ± 0.075 | 0.139 ± 0.077 | NS |
| Brain | 0.465 ± 0.029 | 0.462 ± 0.017 | 0.458 ± 0.022 | NS |
| Lung | 0.172 ± 0.039 | 0.185 ± 0.043 | 0.169 ± 0.037 | NS |

* Compared to Vehicle treatment; ns, not significant

**Figure 12**

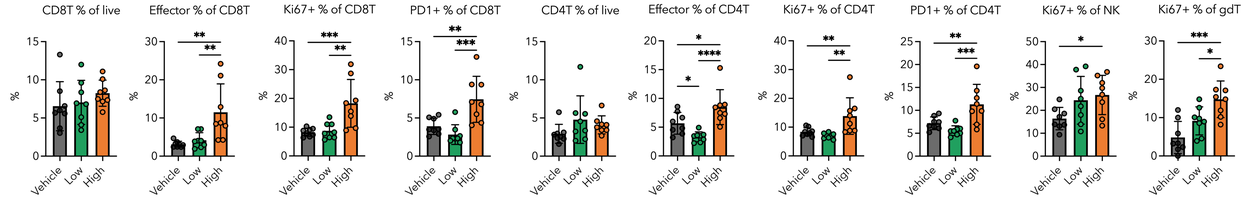


**Figure 12:** Flow cytometry analysis of peripheral blood mononuclear cells (PBMC) isolated from CAN-3110 treated B16-F10-nectin1-hi tumor-bearing mice. N=8 per group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

## Conclusions

- Selective replication of CAN-3110 is conferred by both CDKN2A loss-of-function and nestin expression.
- CAN-3110 demonstrated potent antitumor activity both in vitro and in vivo, highlighting its potential as a promising therapeutic agent for treating melanoma.

**References:**
1. Cancer Res. 2005;65(7):2832-9.
2. Mol Ther Methods Clin Dev. 2020;17:871-893.
3. Nature. 2023;623(7985):157-166.
4. Oncogene. 2008;27(30):4249-54
5. Mol Ther Oncolytics. 2020;18:476-490.

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