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Safety and Efficacy Evaluation of Retroviral Vectors GEN-1018 and GEN-1013 Toward Clinical Translation for *In Vivo* Immuno-gene Therapy and Combined Suicide Gene Therapy

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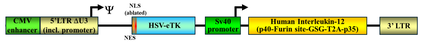
28TH ANNUAL ASGCT MEETING MAY 13-17, 2025 NEW ORLEANS

BACKGROUND

- Interleukin-12 (IL-12) is a heterodimeric pro-inflammatory cytokine that is naturally produced by dendritic cells, macrophages, neutrophils and helper T cells in response to antigenic stimulation. In murine tumor models, IL-12 has been found to engage and stimulate anti-tumor immunity by activating the effector TH1 cells to produce IFN-γ, which is required for the activation of antigen presenting cells, which in turn activate cytotoxic T and NK cells, resulting in tumor clearance [1]. However, potentially lethal toxicity associated with systemic administration of recombinant IL-12 protein precludes its clinical application [11]. Realizing the potential benefits of IL-12-based therapies for solid malignancies requires localized IL-12 expression with only limited systemic exposure to mitigate the associated toxicity.
- The GEN-1013 γ-retrovector (Figure 1) incorporates into actively dividing cell populations, including proliferating tumor cells.

Figure 1. Human GEN-1013 Vector Linear Genome Map
Gen-1013 encodes IL-12 and HSV -eTK

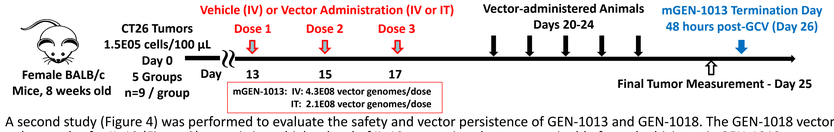


- As human IL-12 is inactive in wild-type mice, a surrogate vector (mGEN-1013) encoding for murine IL-12 is used in immunocompetent BALB/c mice of > 8 weeks of age during IND-enabling nonclinical pharmacology and toxicology evaluations.
- Conversion of the prodrug ganciclovir (GCV) by HSV-eTK protein expressed from the GEN-1013 payload offers a means to release patient-specific tumor antigens from dying tumor cells into a local immune environment as well as a safety mechanism to terminate expression of IL-12 if needed.

METHODS

In one proof-of-concept study (Figure 2), mice dosed with mGEN-1013 either intravenously (IV) or intratumorally (IT) were evaluated for tumor growth rate against a vehicle control across the routes of administration.

Figure 2. Proof of Concept Study Timeline



A second study (Figure 4) was performed to evaluate the safety and vector persistence of GEN-1013 and GEN-1018. The GEN-1018 vector only encodes for IL-12 (Figure 3), permitting a higher level of IL-12 expression than was attainable from the bicistronic GEN-1013 vector.

Figure 3. Human GEN-1018 Vector Linear Genome Map

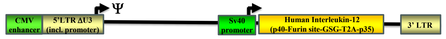
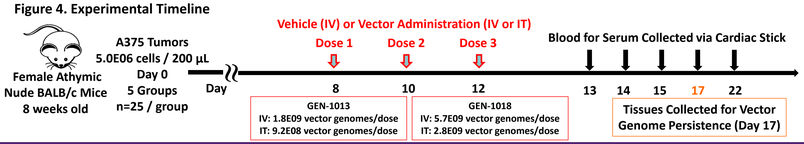


Figure 4. Experimental Timeline



RESULTS

Increased Percentage of Tumors Below Euthanasia Threshold Observed In mGEN-1013-Dosed Immunocompetent BALB/c Mice Versus Vehicle Control

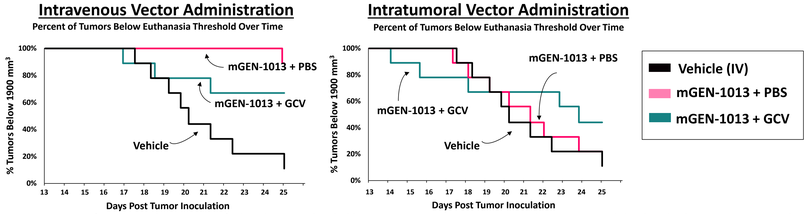


Figure 5. Percent of Tumors Below Pre-Specified Euthanasia Threshold Over Time. Tumors were measured twice per week by digital caliper. Per institutional IACUC guidelines, euthanasia is required if tumors exceed 2000 mm³. Individual animals were therefore classified based on their tumor size relative to a pre-specified maximum tumor volume (1900 mm³) reached before euthanasia was implemented. n=9 for all groups. Ordinary one-way ANOVA with Dunnett's multiple comparisons test was performed on tumor volumes of mice sacrificed on Day 25 (n=6-9 per group), comparing each group to the vehicle control: mGEN-1013 + PBS (IV) p = 0.002**, mGEN-1013 + GCV (IV) p = 0.015*, mGEN-1013 + PBS (IT) p=0.826, mGEN-1013 + GCV (IV) p=0.043*.

RESULTS

Increase In Circulating Activated Effector T and NK Cells Following mGEN-1013 Dosing

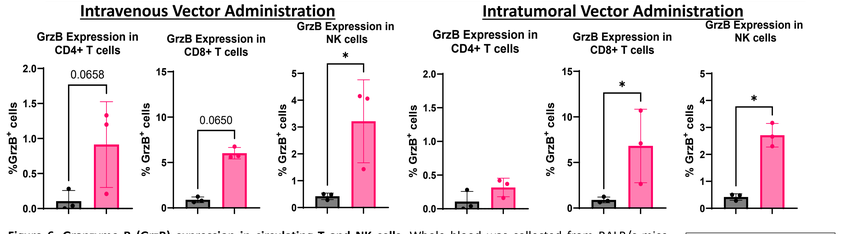


Figure 6. Granzyme B (GrzB) expression in circulating T and NK cells. Whole blood was collected from BALB/c mice euthanized on day 26 via cardiac stick (n=3 per group used for flow cytometry). Intravenous and intratumoral administration of mGEN-1013 showed increased granzyme B (GrzB) expression in CD4+ and CD8+ T cells and NK cells in the blood as seen by flow cytometry. GrzB+ T cells were gated from live, CD45+CD3+ cells that were either CD4+ or CD8+. GrzB+ NK cells were gated from live, CD45+CD3$_{neg}$CD49b+ cells. Ordinary one-way ANOVA with Dunnett's multiple comparisons test was performed on all graphs, * p < 0.05.

Transduced T Cells Are Distributed Within Tumors After mGEN-1013 Dosing

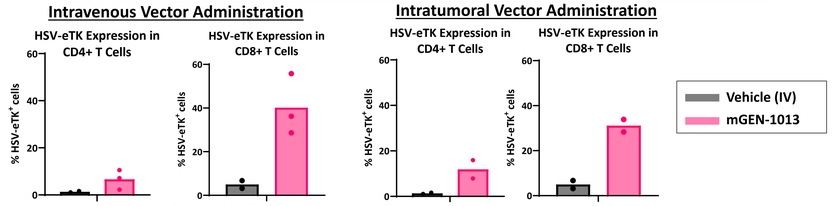


Figure 7. HSV-eTK expression in tumor-infiltrating lymphocytes. Intravenous and intratumoral administration of mGEN-1013 showed increased HSV-eTK expression in CD4+ and CD8+ T cells in the tumor microenvironment as seen by flow cytometry. HSV-eTK+ cells were gated from live, CD45+CD3+ cells that were either CD4+ or CD8+. Tumors were dissociated into single-cell suspension using the Miltenyi Mouse Tumor Dissociation Kit (catalog # 130-096-730), which is optimized for high yield of tumor cells and tumor infiltrating lymphocytes.

Differing Vector Genome Persistence Observed for mGEN-1013 Based on Route of Administration in Immunocompetent Mice

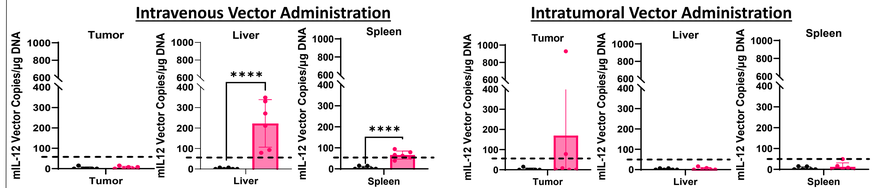


Figure 8. Vector Persistence of mGEN-1013 in tumor, liver, and spleen. Genomic DNA was extracted from tumor, liver, and spleen tissues from all BALB/c mice euthanized on day 26 (n=6 per group). ddPCR was performed using primers for vector-specific mouse codon-optimized IL-12 p40 sequence, using mouse RPP30 as the reference gene. Dashed line indicates assay LOQ (50 copies/ug DNA). Ordinary one-way ANOVA with Dunnett's multiple comparisons test was performed on all graphs, **** p < 0.0001.

CONTACT

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GenVivo is a private clinical stage company with innovative patented off-the-shelf genetic medicine platforms for cancer immunotherapies.

RESULTS

Vector Genome Persistence of Human IL-12-Encoding Vectors (GEN-1013 & GEN-1018) in Immunodeficient Athymic BALB/c Mice Is Dependent on Route of Administration

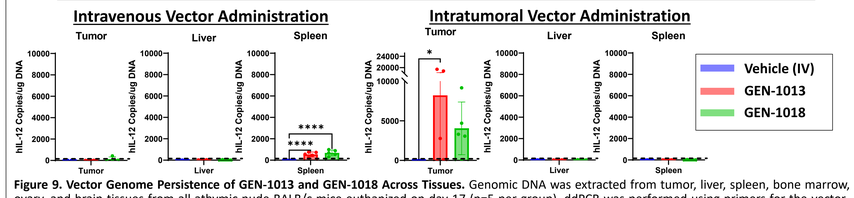

Figure 9. Vector Genome Persistence of GEN-1013 and GEN-1018 Across Tissues. Genomic DNA was extracted from tumor, liver, spleen, bone marrow, ovary, and brain tissues from all athymic nude BALB/c mice euthanized on day 17 (n=5 per group). ddPCR was performed using primers for the vector-specific human codon-optimized p35 subunit of IL-12, using human RPP30 as the reference gene for tumor tissue and mouse RPP30 for all other tissues. Dashed line indicates assay LOQ (50 copies/ug DNA). No significant distribution was found in ovaries, bone marrow or brain tissue. Ordinary one way-ANOVA with Dunnett's multiple comparisons test was performed. * p < 0.05; **** p < 0.0001.

Human GEN-1013 and GEN-1018 in Immunodeficient Athymic BALB/c Mice Show Post-Administration Serum IL-12 Levels Below Maximum Tolerated Level in Primates and Humans

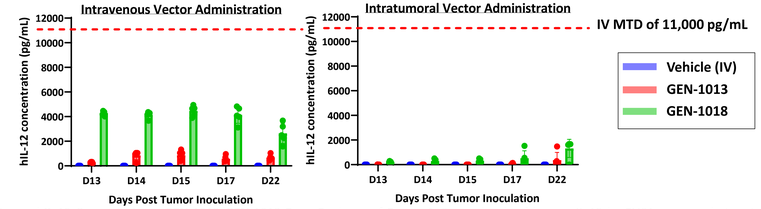

Figure 10. Serum hIL-12 Concentrations GEN-1013/GEN-1018 Dose Regimen of Figure 4. Serum levels of human IL-12 by ELISA measured at various timepoints following vector IV dosing were well below the C_{max} on IV dosing at the maximum tolerated dose (MTD) in cancer patients (~11,000 pg/mL; Atkins 1997 CCR) or subcutaneous dosing (~700 pg/mL; Motzer 1998 CCR). Dashed line indicated IV MTD of 11,000 pg/mL.

CONCLUSIONS

- 89% of mGEN-1013 IV-treated animals (in the absence of GCV) reached the predetermined study endpoint compared to 11% of the vehicle control animals.
- Tumor growth rate reduction was dependent on GCV prodrug treatment in IT-dosed animals but not required for IV-dosed animals.
- Notably, immune activation was observed with both IV and IT administration of mGEN-1013, correlating with the presence of intratumoral GEN-1013-transduced T cells.
- Observed differences in vector genome persistence were consistent with route of administration:
  - IV administration resulted in vector genomes localized to spleen and liver
  - IT administration resulted in localization to the tumor, with no significant presence in liver or spleen
- An immunodeficient model to maximize circulating IL-12 levels showed that such levels remained below human and primate maximum-tolerated levels after IV or IT administration of human GEN-1013. Serum levels from the IL-12-only GEN-1018 vector, while markedly elevated, remained confined to this tolerable range. GEN-1013 administration resulted in production of IL-12 to stimulate antitumor immune responses without approaching any level of IL-12 toxicity.
- GEN-1013 administration is safe and well-tolerated in BALB/c mice at an efficacious dose level within these studies. Comprehensive pharmacology and toxicology assessments to optimize GEN-1013 dose level and schedule are in progress.

ACKNOWLEDGEMENTS

This work was made possible by the work of the Pre-Clinical, R&D, and PSE departments at GenVivo, Inc., as well as the staff at Charles River Accelerator and Development Lab – Thousand Oaks.

REFERENCES

Please refer to published abstract ID AMA1956, and as cited explicitly herein.