NK CELLS ENGINEERING USING ADVANCED LENTIVIRAL VECTORS QUALITY AND DESIGN

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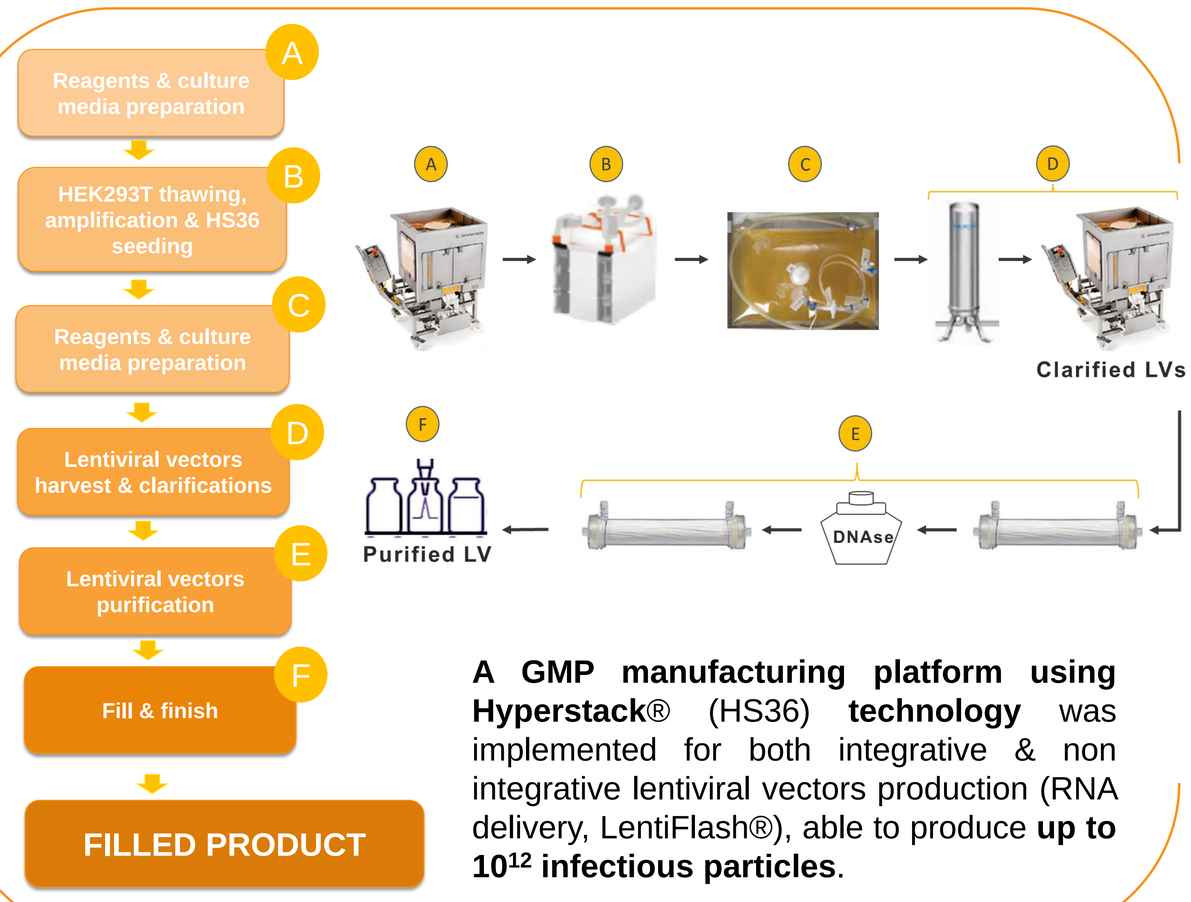
A. New challenge for Immunotherapy

The emergence of targeted immunotherapy, especially chimeric antigen receptor **(CAR) T-cell therapy**, has opened new possibilities, demonstrating tremendous success for patients with lymphoblastic leukemia. **Natural Killer** (NK) cells are other key immune effector cells which, contrary to T cells, do not require antigen priming and are at a low risk of Graft-Versus-Host Disease (GVHD), therefore offering the potential of an allogenic "off-the-shelf" therapeutic product. Nevertheless, primary NK cells exhibit a high resistance to lentiviral transduction, hampering transgene expression and consequently the generation of **CAR-NK** cells.

Pre-clinical and clinical CAR-NK cells manufacturing requires to be performed using the same process. Here we show a manufacturing process of highly purified and concentrated third generation lentiviral vectors (LVs), available for a **continuum going from Discovery to Clinic phases**.

Integrating a control plan, allowing for the development and for the good manufacturing practices (**GMP**) production of **custom lentiviral batches** dedicated to clinical applications. **Human primary NK cells**, previously activated by artificial antigen-presenting cells (APC) **are successfully transduced** with such **highly purified and concentrated Lentiviral vectors** prior to be evaluated by in vitro cytotoxic assays and in vivo engraftments into mouse model.

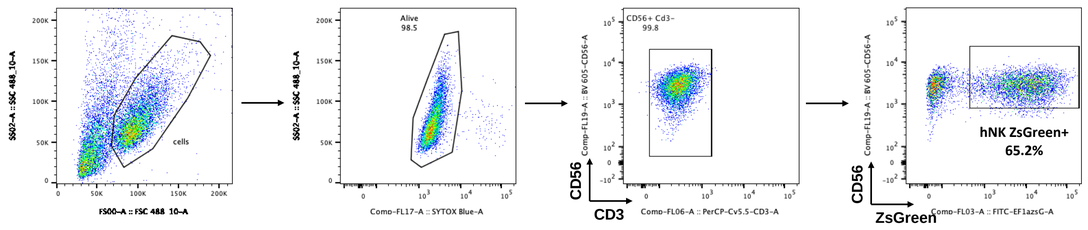
B. LVs Manufacturing Process flowchart



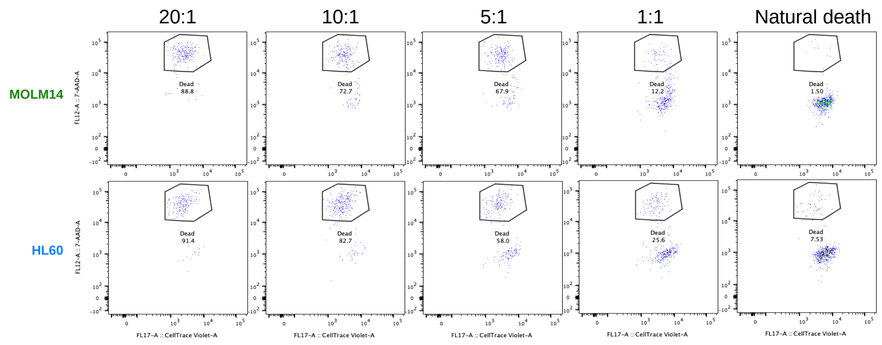
A GMP manufacturing platform using Hyperstack® (HS36) **technology** was implemented for both integrative & non integrative lentiviral vectors production (RNA delivery, LentiFlash®), able to produce **up to 10^{12} infectious particles**.

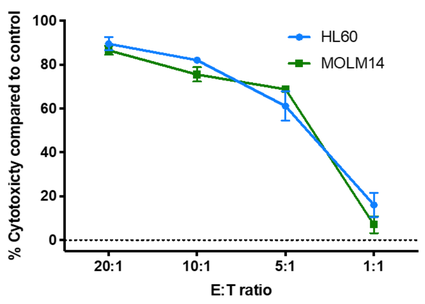
C. Ex vivo Transduction of primary hNK cells

hNK CD56+ CD3- Cord blood cells were cultivated using the G-Rex® system (Wilsonwolf), in presence of IL-2, IL-15 and IL-21 and activated with artificial APCs. hNK cells were transduced twice at MOI 50 with a highly purified and concentrated Lentiviral vectors ILV-EF1-ZsGreen:



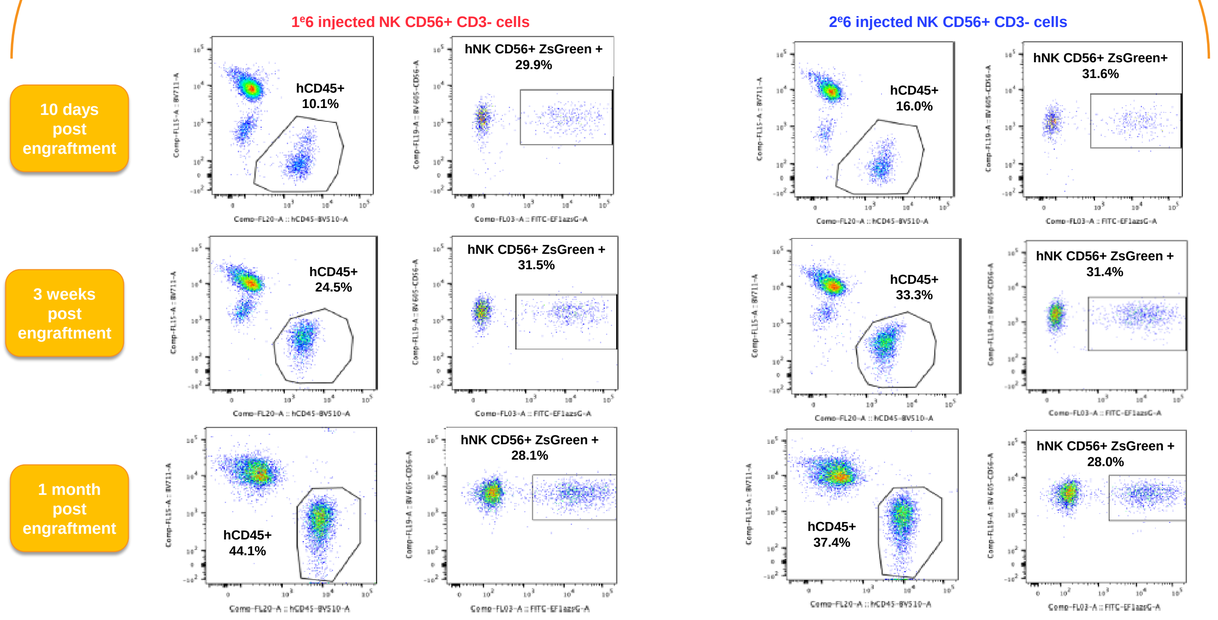
Transduced human primary NK cells can efficiently kill HL60 and MOLM14 target cells *in vitro* (17h incubation at various Effector:Target cells ratio):

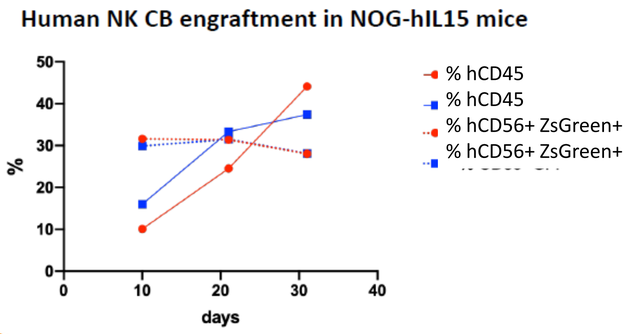




D. Engraftment of stably transduced hNK cells

hNK cells modified *ex vivo* are efficiently maintained *in vivo for* up to one month post engraftment :
After activation with artificial APCs, NK CD56+ CD3- Cord blood cells were transduced twice at MOI 50 and then reactivated again prior to injection into NOG-hL15 mice (Taconic). PBMC are analysed:





E. CONCLUSIONS

In this collaborative work, we show that the use of **highly purified and concentrated** self-inactivating **lentiviral vectors** in combination with an optimized transduction protocol, **allows** up to **65% of transduced** human cord blood derived **NK cells**. Additionally, we show that transduction does not lead to viability nor phenotypic alterations of the transduced NK cells. Our approach not only achieves high transduction efficiency, leading to **strong and stable transgene expression**, but also **preserves** the **cytotoxic function** of the **NK cells**, *in vitro* and *in vivo*.

Many obstacles exist for **clinical development** of a **CAR-based cellular therapy** product, which **requires efficient** and **safe delivery technologies**, as well as gene expression level and duration tailoring. It's **possible to achieve** this, through the use of delivery tools, which **allow** highly **efficient gene transfer while maintaining** transduced **cell viability** and **phenotype**. Here we propose a **novel method** allowing for the generation and production of **lentiviral vector engineered primary NK cells**, thus circumventing the problem of poor autologous CAR-T cell efficiency and gamma retrovirus associated risks.

This work lays the groundwork for novel cellular therapies based on lentivirally transduced primary NK cells. All these factors, as well as the **ability to produce lentiviral vectors using Flash Therapeutics' GMP compliant production platform**, offer additional safety considerations for clinical development and human use.

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