Monocyte-Based Transport of HSV-1 Oncolytic Viruses for Enhanced Acute Myeloid Leukemia Targeting

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Introduction

In light of the urgent need for innovative therapies against acute myeloid leukemia (AML), where current treatments fail to target the protective bone marrow niche effectively, the present research investigates monocyte-mediated delivery of oncolytic Herpes Simplex Virus type 1 (oHSV-1) as a novel virotherapy approach (Figure 1).

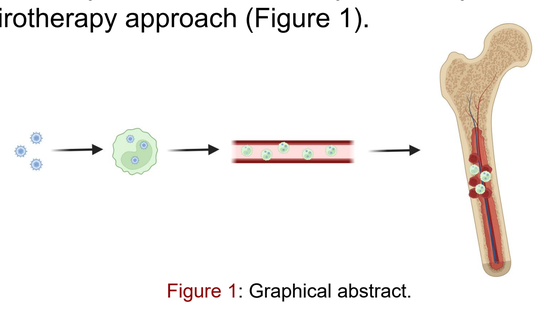


Figure 1: Graphical abstract.

Materials & Methods

Δγ34.5/ΔUs12/mir124/mCherry-oHSV1

Δγ34.5/ΔUs12/mir124/mCherry-oHSV1 (oHSV1-mCherry; Figure 2) is an oncolytic HSV-1 virus, obtained from 17+ strain, presenting the following modifications:

- Deletions of γ34.5 genes;
- Deletion of Us12 gene;
- Insertion of mir124 target sequences in UL29 promoter;
- Insertion of a gene expression cassette, encoding the protein mCherry under the CMV Immediate early promoter, in UL55-56.

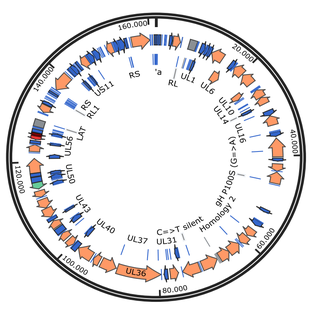


Figure 2: Representative map of oHSV1-mCherry.

Primary Human Autologous Monocytes Isolation and Infection

Human autologous monocytes are isolated from buffy coats of healthy donors, provided by University Hospital of Padua, through Ficoll gradient separation followed by CD14+ cell selection by EasySep™ Human CD14 Positive Selection Kit II (Stemcell).
The day after purification, they are labeled with CellTracker™ Green (Invitrogen) and are infected with oHSV1-mCherry, at a multiplicity of infection (MOI) of 3.

Analysis of monocytes ability to transmit viral infection to AML cells

Labeled and infected monocytes are co-cultured with MV-4-11 cells and monitored for viral replication in the following days.

Evaluation of monocytes tropism for MV-4-11 cells

Transwells Permeable Supports are used to assess monocytes migration towards AML cells.

Evaluation of monocytes capacity to migrate inside hydrogels and transmit viral infection to MV-4-11 cells

MV-4-11 cells are cultivated in Gelatin Methacryloil (GelMA) 7%, an hydrogel mimicking the bone marrow extracellular matrix, and are treated with labeled and infected monocytes (Figure 3). Monocytes entry and viral transmission are evaluated through fluorescent microscopy.

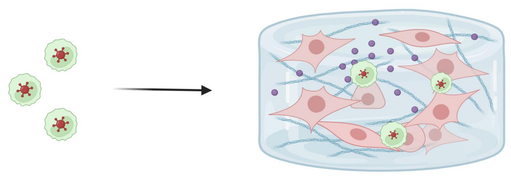


Figure 3: Schematic representation of GelMA 7% treated with infected and labeled monocytes.

Conclusions

Monocytes effectively act as carriers of HSV-1 oncolytic virus, preserving migratory capacity, showing tropism toward AML cells, and reducing leukemic cell viability through viral transmission.
GelMA 7% provided a permissive 3D bone marrow–like microenvironment, maintaining MV-4-11 viability while enabling monocyte infiltration and efficient HSV-1 transmission.
These findings support autologous monocytes as “Trojan horse” carriers and highlight the value of biomimetic 3D platforms for preclinical validation of cell-mediated virotherapy.

Results

oHSV1-mCherry replicates and kills MV-4-11 cells

At 48 hours post-infection, diffuse red fluorescence indicates active viral replication in AML cells. This replication reduces cells viability, showing the oHSV1-mCherry ability to kill MV-4-11 cells (Figure 4).

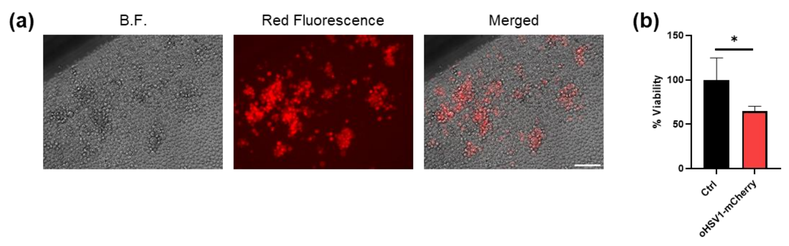


Figure 4: oHSV1-mCherry is observed at 48h post-infection (a) in MV-4-11 cells (MOI = 10 PFU/cell). The MTT assay (b), performed at 72 hours post-infection, demonstrates the virus killing ability compared to the control group (Ctrl).

Monocytes transmit viral infection to MV-4-11 cells, impacting their viability

At 13 days after introducing infected monocytes,viral replication is observed in the majority of leukemia cells, as evidenced by the diffuse red fluorescence. This viral replication negatively affects cells viability, confirming oHSV1-mCherry ability to kill leukemia cells (Figure 5).

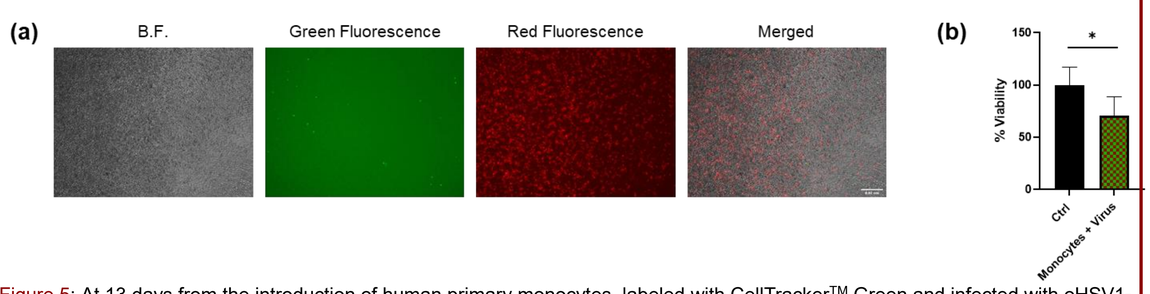
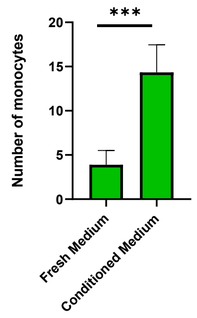


Figure 5: At 13 days from the introduction of human primary monocytes, labeled with CellTracker™ Green and infected with oHSV1-mCherry (5000 monocytes/well), efficiently transmit the viral infection to MV-4-11 cells (a). The MTT assay (b), performed at the same time point, hilightes a reduction in viability in the treated samples (Monocytes + Virus), compared to the control groups (Ctrl).

Monocytes have tropism for AML cells

The number of monocytes recruited to wells containing medium conditioned by MV-4-11 is statistically higher than that observed in the control, confirming monocytes ability to migrate towards AML cells (Figure 6).

Figure 6: After 30 minutes from the introduction of human primary monocytes labeled with CellTracker™ Green (60.000 monocytes/well), at least 3 images for each well are captured. A statistical difference in the number of recruited monocytes between the control (Fresh Medium) and the Conditioned Medium groups is observed.



MV-4-11 seeded in GelMA 7% are viable

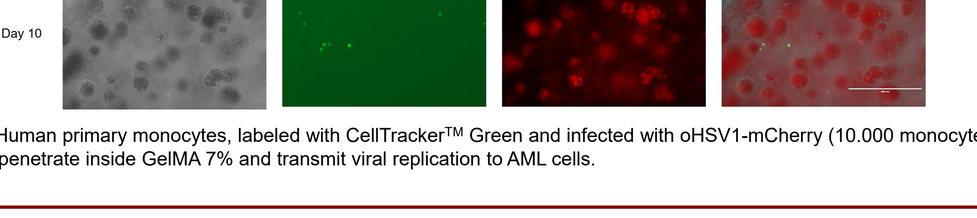


Figure 7: MV4-11 cells mantained in GelMA 7%, labeled with Hoecst, Calcein AM and Propidium Iodide at Day 10 post-introduction.

Monocytes migrate inside GelMA 7% and transmit viral infection to AML cells

In days following monocytes introduction, these cells are identified within the GelMA structure, demonstrating their capacity to reach MV-4-11 cells even in the presence of extracellular matrix. Moreover, over time, a progressive increase in red fluorescence is observed, supporting the notion that monocytes transmit oHSV1-mCherry to AML cells (Figure 8).

Figure 8: Human primary monocytes, labeled with CellTracker™ Green and infected with oHSV1-mCherry (10.000 monocytes/well), efficiently penetrate inside GelMA 7% and transmit viral replication to AML cells.

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