TROPISM OF ONCOLYTIC VACCINIA VIRUS CONSTRUCTS FOR HUMAN MONONUCLEAR CELL SUBSETS

Nanhai G. Chen[1,2], Mehmet O. Kilinc[1], Qian Zhang[1,2], Jason Aguilar[1], Desislava Tsoneva[3], Uma Patel[1], Boris Markosian[4], Maysam Pessian[4], Boris Minev[1,4,5], Aladar A. Szalay[1,2,3]

[1]Genelux Corporation, San Diego, CA
[2]Department of Radiation Oncology, University of California San Diego
[3]University of Wurzburg, Wurzburg, Germany
[4]Moores UCSD Cancer Center
[5]Division of Neurosurgery, University of California San Diego

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Presenter Disclosure Information

Boris Minev, M.D.

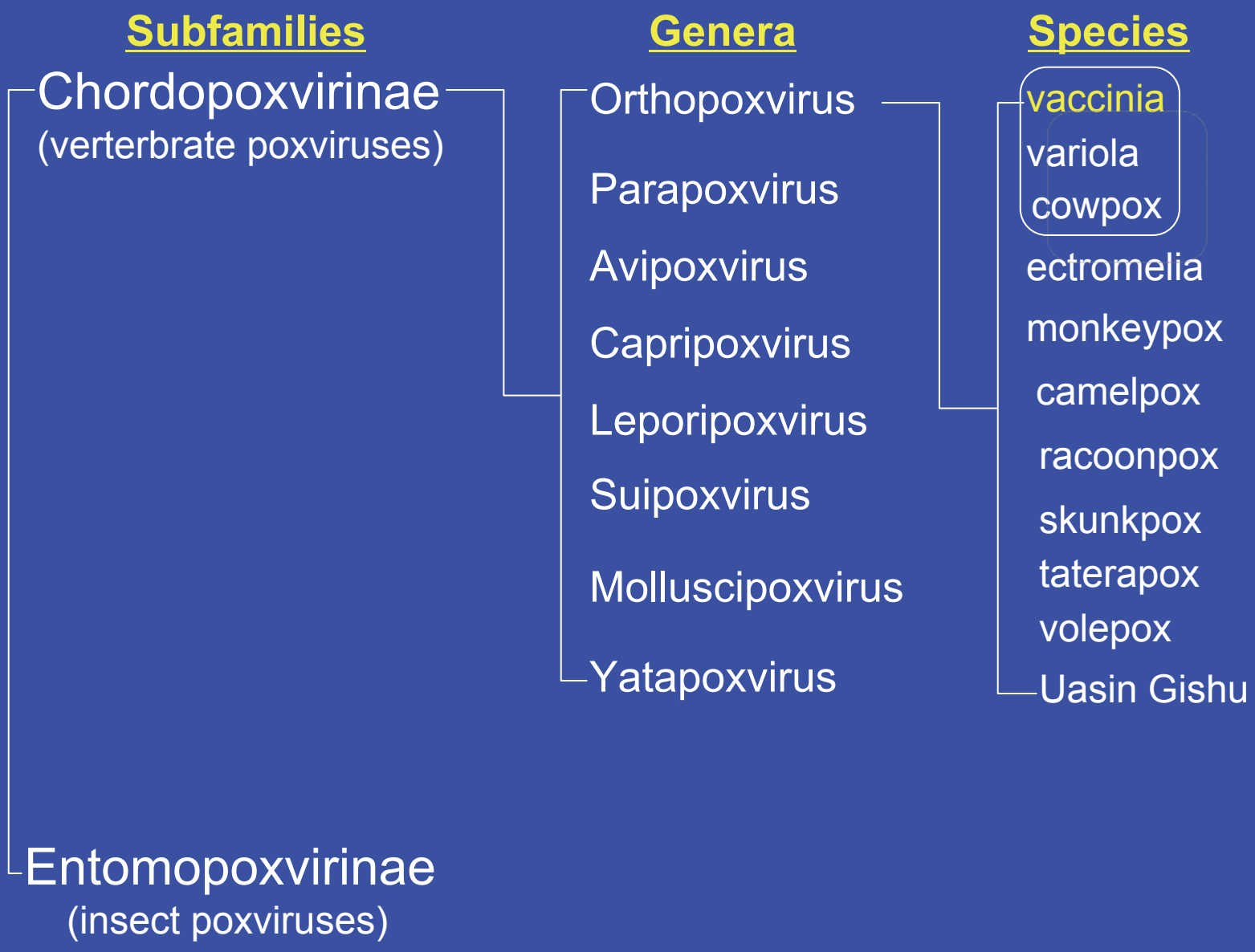
The following relationships exist related to this presentation:

GENELUX CORPORATION: Received Salary as an Employee

Outline

- **Vaccinia Virus (VACV)**
- **Oncolytic VACV Constructs**
- **VACV Tropism (Healthy Donor-derived PBMC)**
  - Adsorption
  - Infection
  - Replication
- **Targeting CMML (Patient-derived PBMC)**

Family Poxviridae



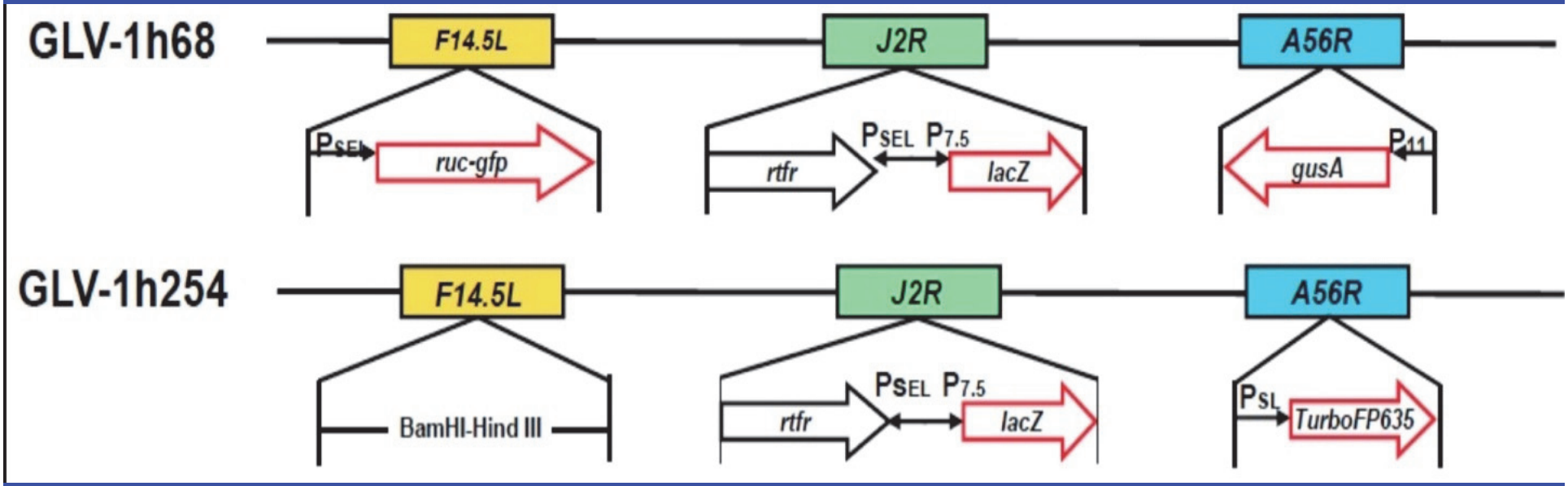
Vaccinia Virus

- A large, stable, enveloped virus (360x270x250 nm)
- A single linear double-stranded DNA genome of ~192 kb
- Insertion capacity: at least 25 kb
- Infects most mammalian cell types
- Does not cause any known human disease
- A cytoplasmic site of replication
- Used extensively as smallpox vaccine in millions of people

Wild-type Vaccinia Strains

- NYCBOH – used in US and West Africa
- Western Reserve (WR) – derived from NYCBOH
- Copenhagen – Northern Europe
- Tian Tan – used in China
- Ankara – Turkey
- MVA – derived from Ankara strain
- Lister – developed at the Lister Institute in the UK
- L.I.V.P. – **L**ister - **I**nstitute of **V**iral **P**reparations (Moscow, Russia)

Oncolytic VACV Constructs



Zhang et al. Cancer Research 67: (20) 10038-10046, 2007

VACV Adsorption Experiments

TurboFP635 Fluorescent Protein

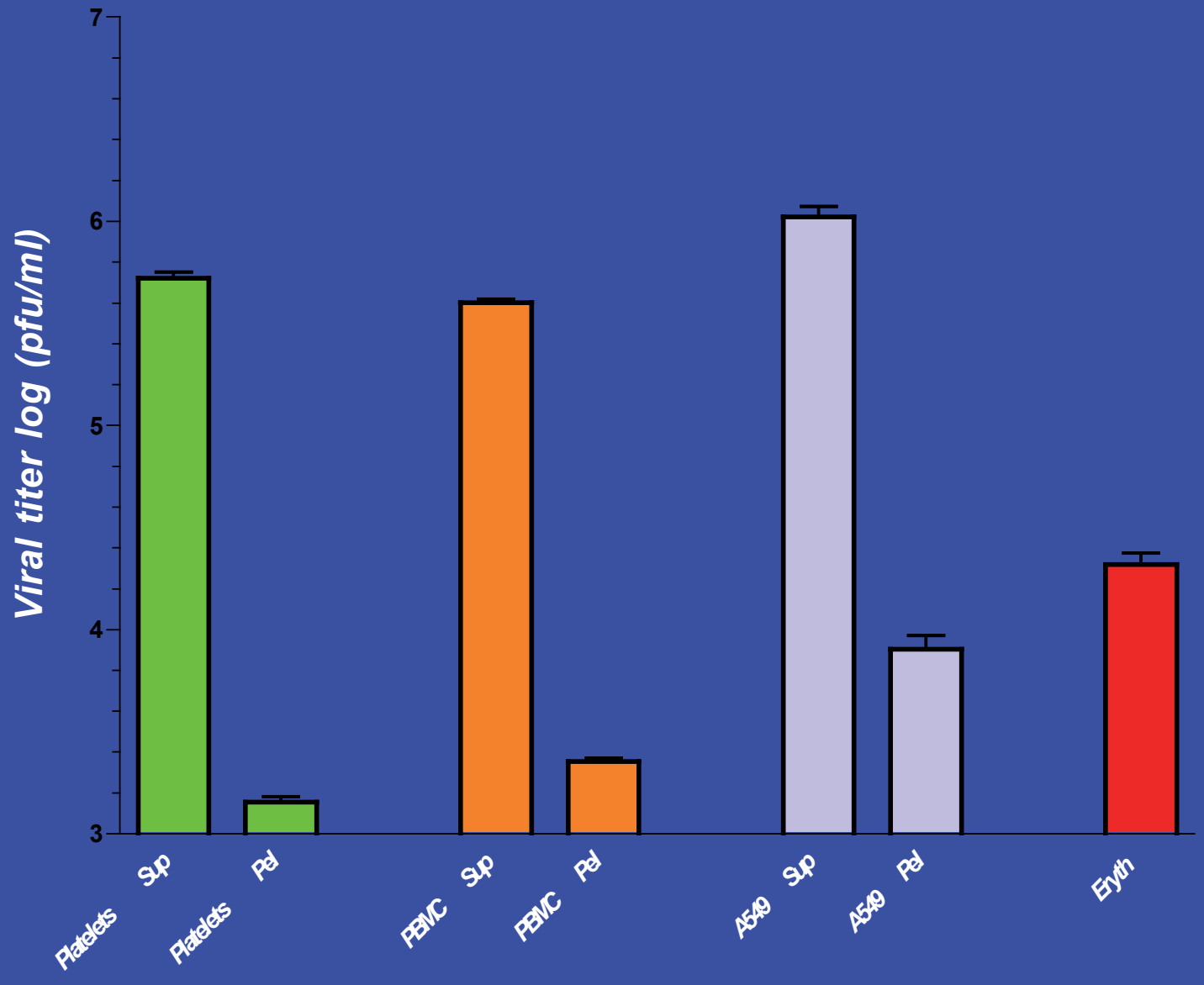
- Super bright far-red fluorescence (Excitation 588nm, Emission 635nm)
- Fast maturation, high photo stability
- Proven suitability to generate stably transfected cell lines
- Fluorescent signal is easily distinguished from background fluorescence
- Recommended for cell and organelle labeling in auto-fluorescent environment, multicolor applications and whole body imaging

- **GLV-1h254 (LIVP-SL-TurboFP) adsorption to A549**
  - A549 with EDTA 50 mM
  - Incubation for 1 Hour at 4^{o}C
  - Titers for supernatants and pellets

- **GLV-1h254 (LIVP-SL-TurboFP) adsorption to Blood Cells**
  - Blood with EDTA 50mM
  - Incubation for 1 Hour at 4^{o}C
  - Blood fractions: Platelets, PBMC and Erythrocytes
  - Titers for supernatants and pellets in each fraction

VACV Adsorption Experiments

GLV-1h254



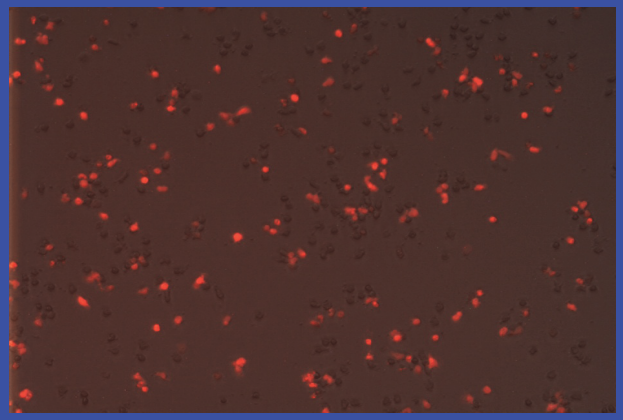
VACV Infection of PBMC Subsets

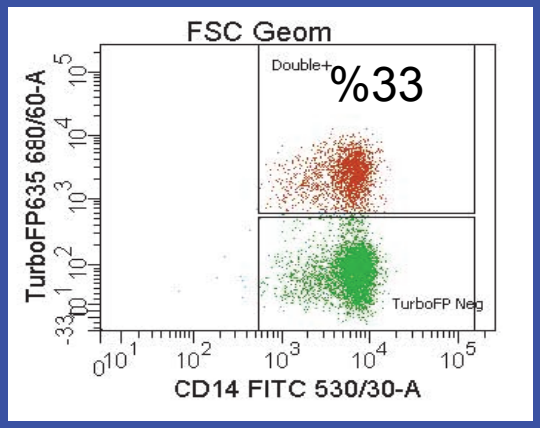
- **PBMC Fractions Isolated with Miltenyi Beads (using AutoMACS):**
  - Monocytes – CD14+
  - B Lymphocytes – CD19+
  - NK Cells – CD56+
  - T cells – CD3+

- **VACV Constructs Used**
  - GLV-1h68 (Psel, GFP) – LIVP Backbone
  - GLV-1h254 (Psl, TurboFP635) – LIVP Backbone
  - GLV-2b372 (Psel, TurboFP635) – LIVP 1.1.1. Backbone
  - GLV-0b347 (Psel, TurboFP635) – WR Backbone

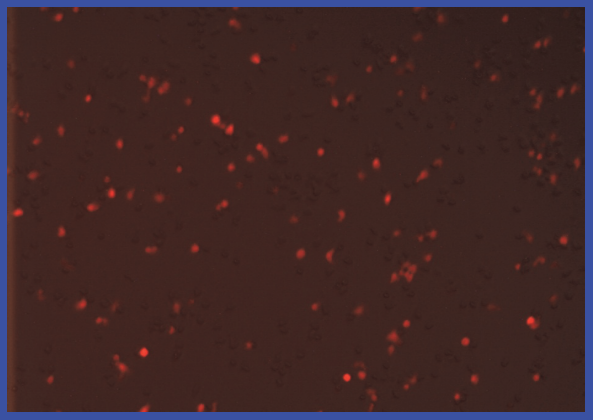
VACV Infection of Monocytes

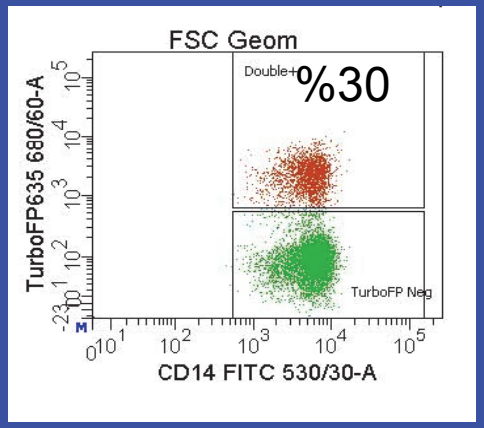
GLV-2b372; MOI:1



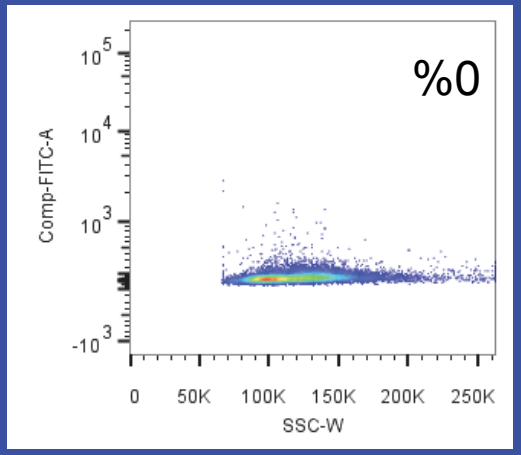


GLV-0b347; MOI:1





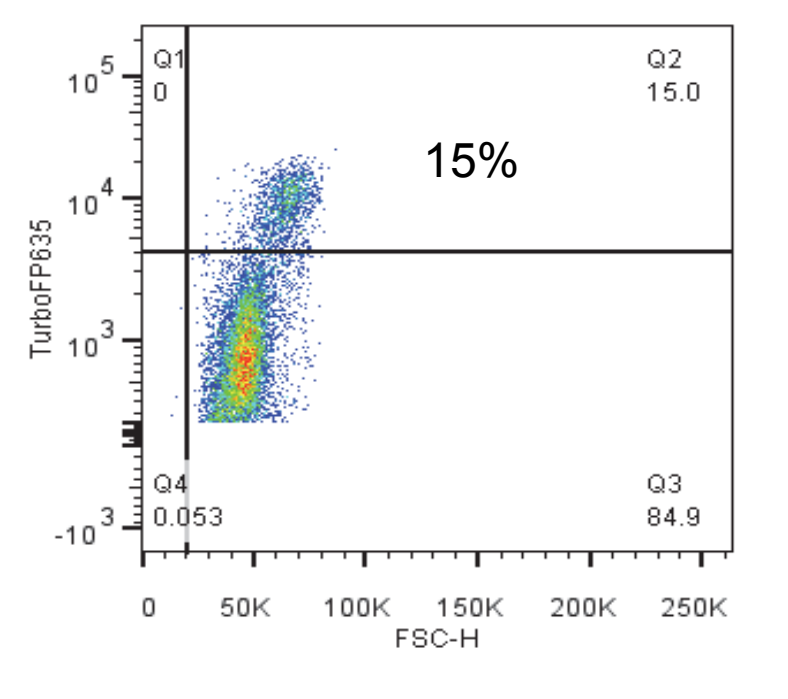
GLV-1h68; MOI:1



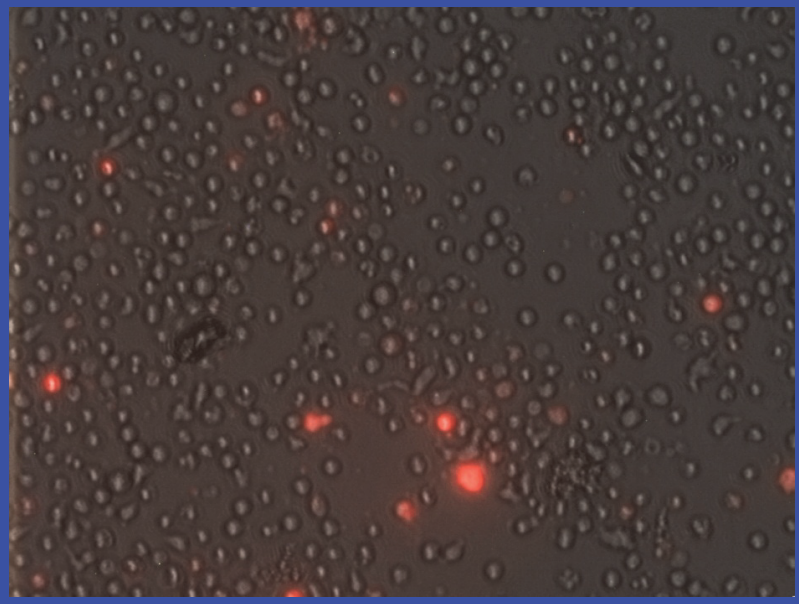
VACV Infection of B Lymphocytes

GLV-2b372; MOI:1

Flow Cytometry



Fluorescent Microscopy

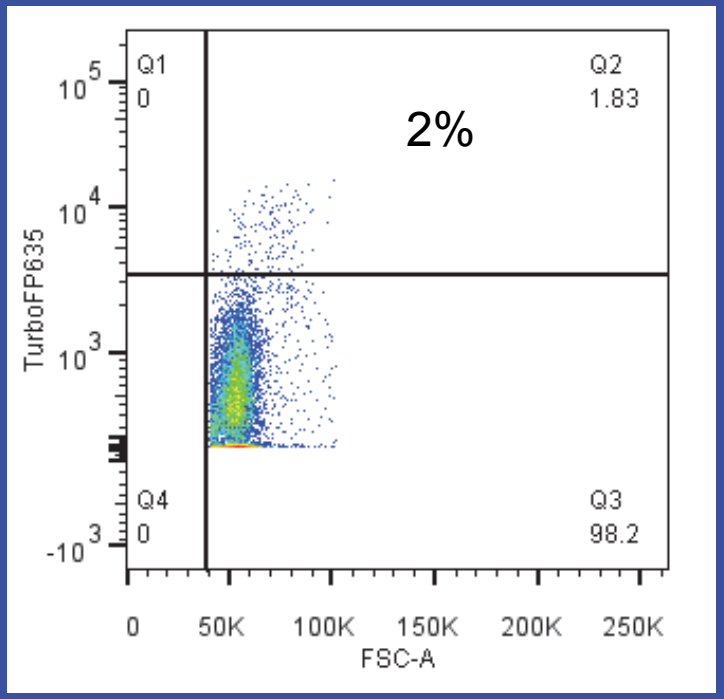


Total # of cells: 701
Total # of infected cells: 99
Percent of infected cells: 14.1 %

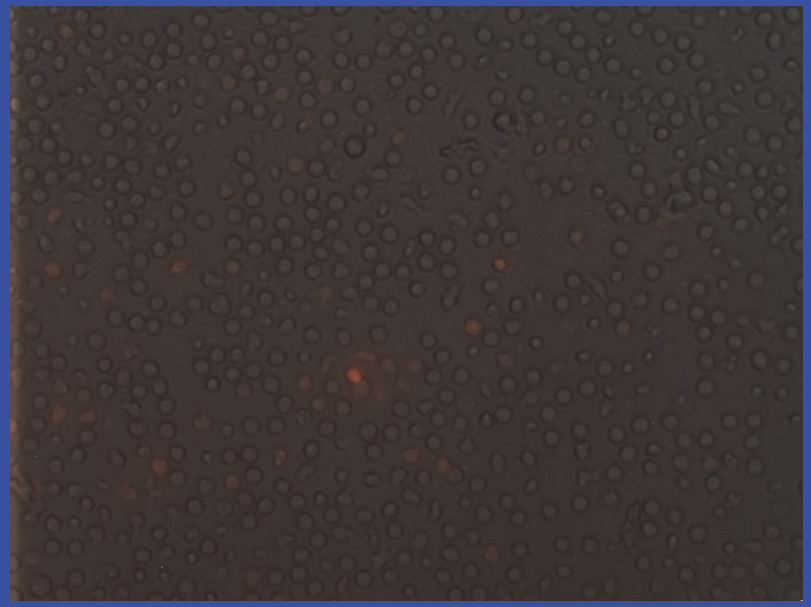
VACV Infection of NK Cells

GLV-2b372; MOI:1

Flow Cytometry



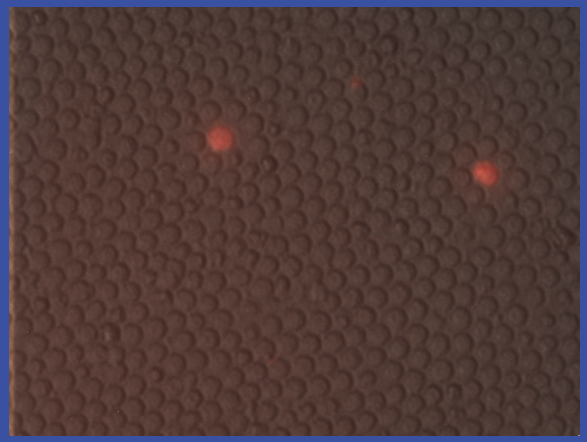
Fluorescent Microscopy

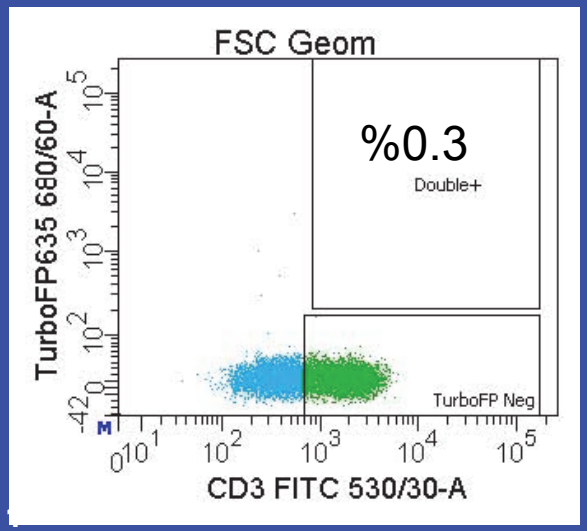


Total # of cells: 1111
Total # of infected cells: 66
Percent of infected cells: 5.9 %

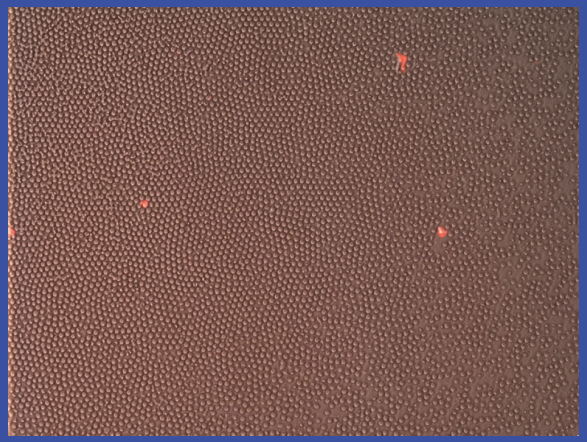
VACV Infection of T Lymphocytes

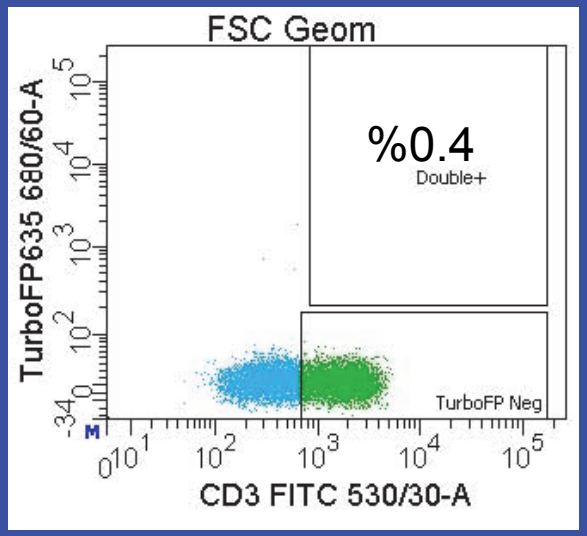
GLV-2b372; MOI:1



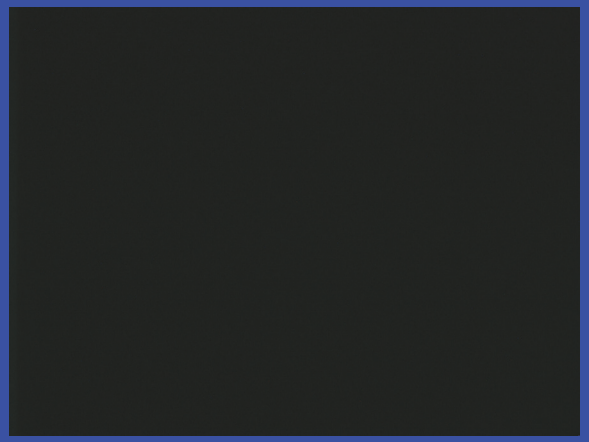


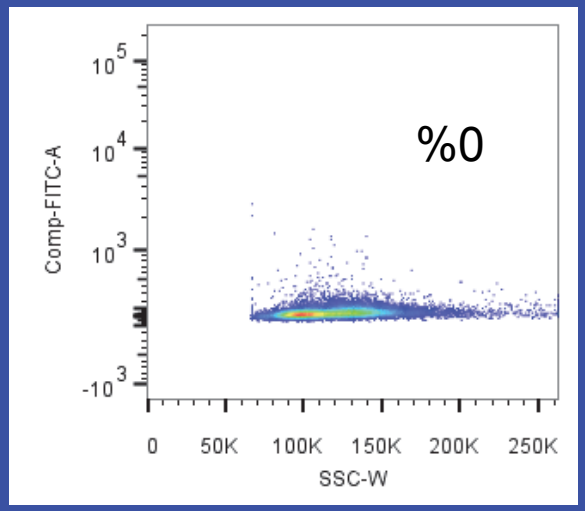
GLV-0b347; MOI:1





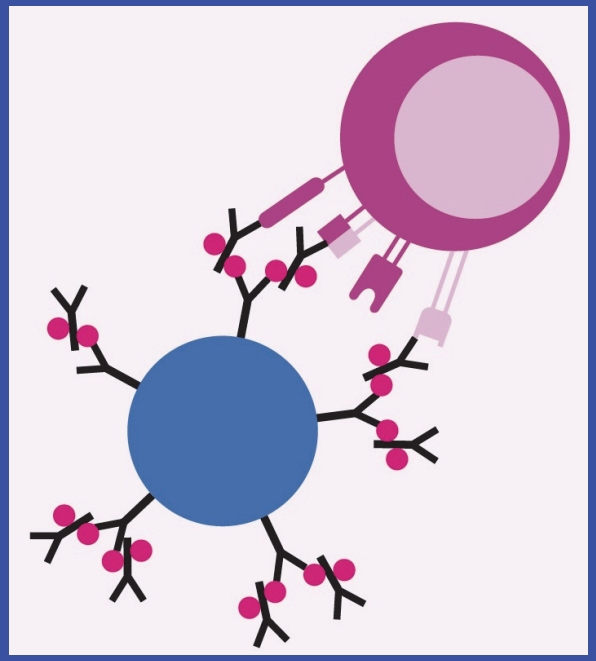
GLV-1h68; MOI:1



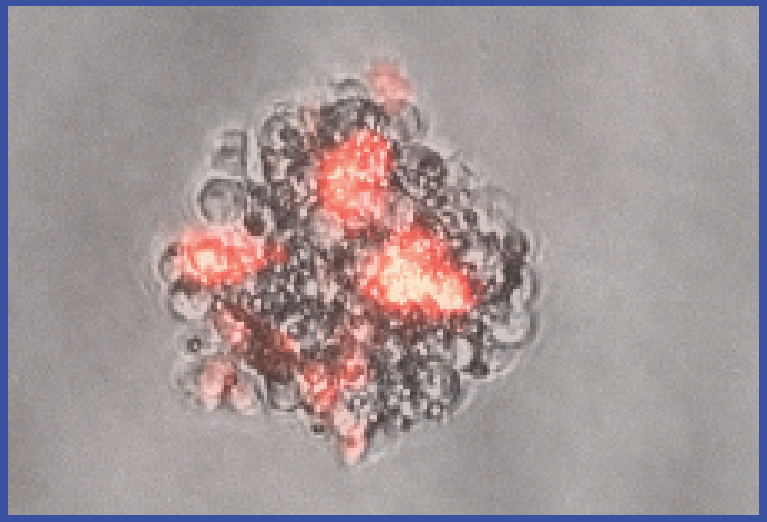


VACV Infection of Activated T cells

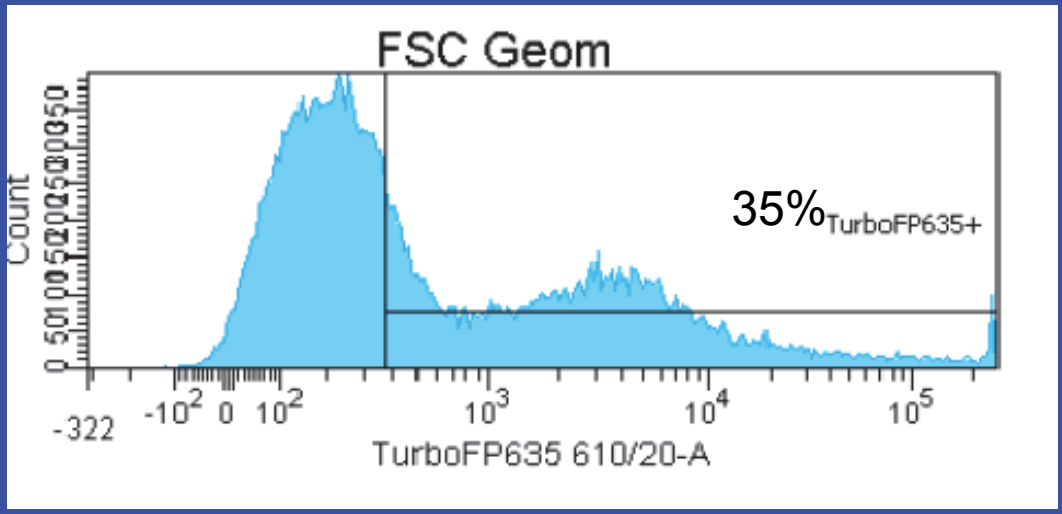
- **Activated T Cells Generated with Miltenyi Beads**
  - Pan T Cell Isolation Kit Used – Negative Selection
  - Resulting T Cells Activated with MACSiBeads
  - T cells activation confirmed with CD25/CD69 staining

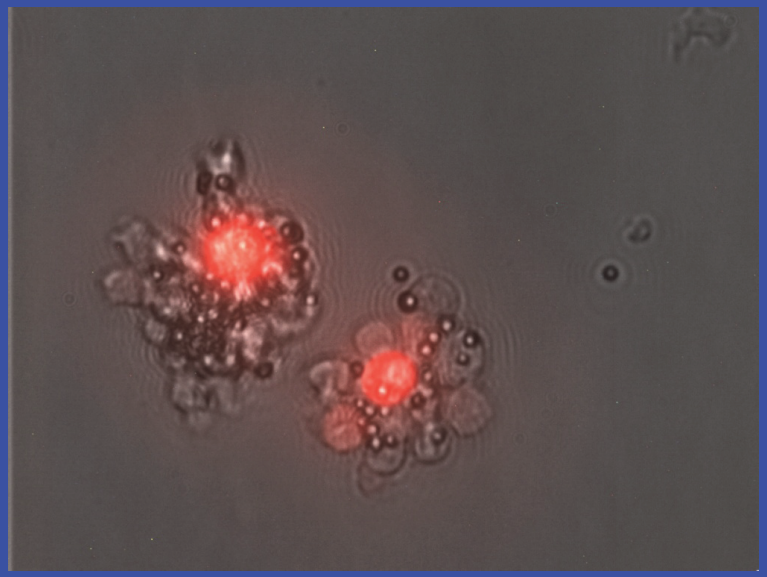


VACV Infection of Activated T Cells

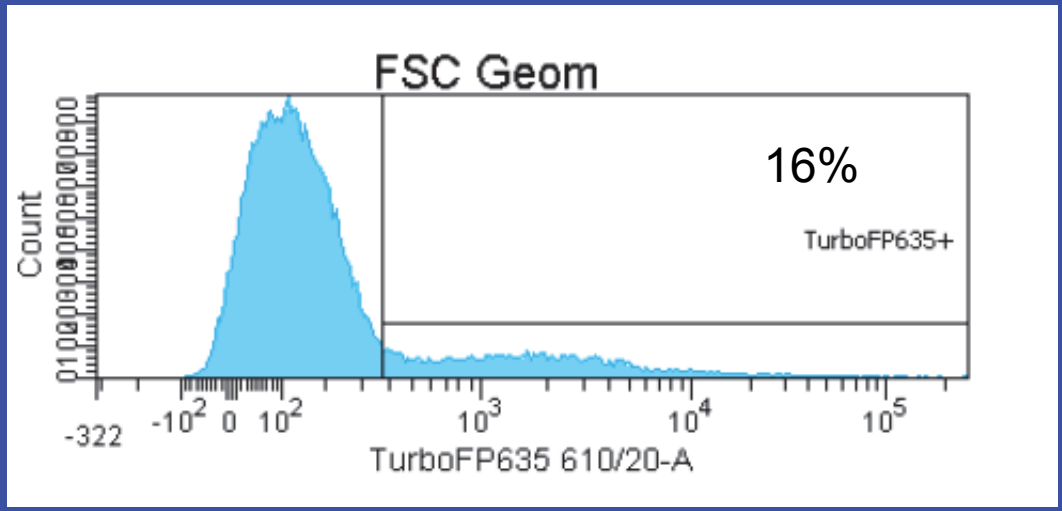


GLV-2b372; MOI:1

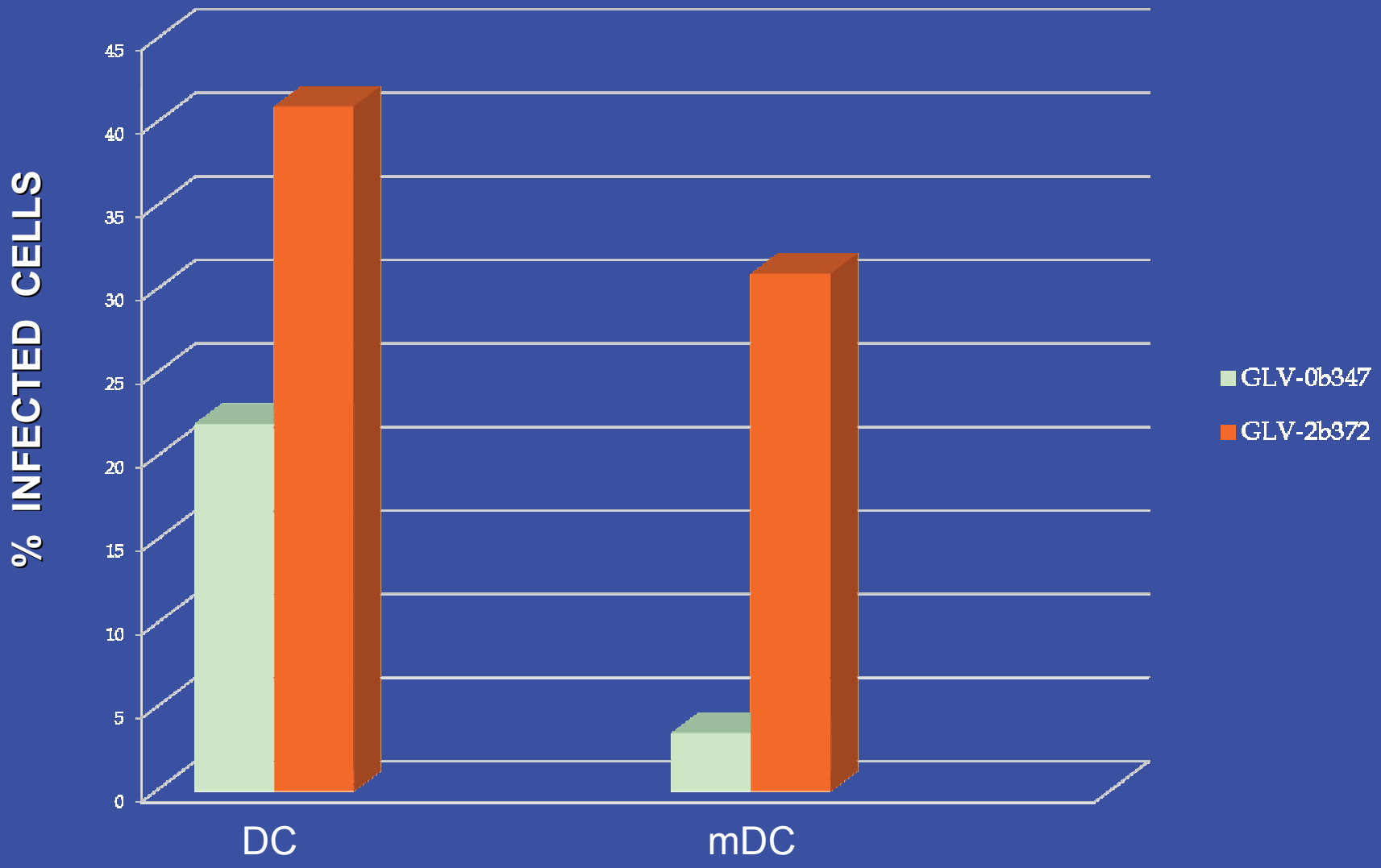




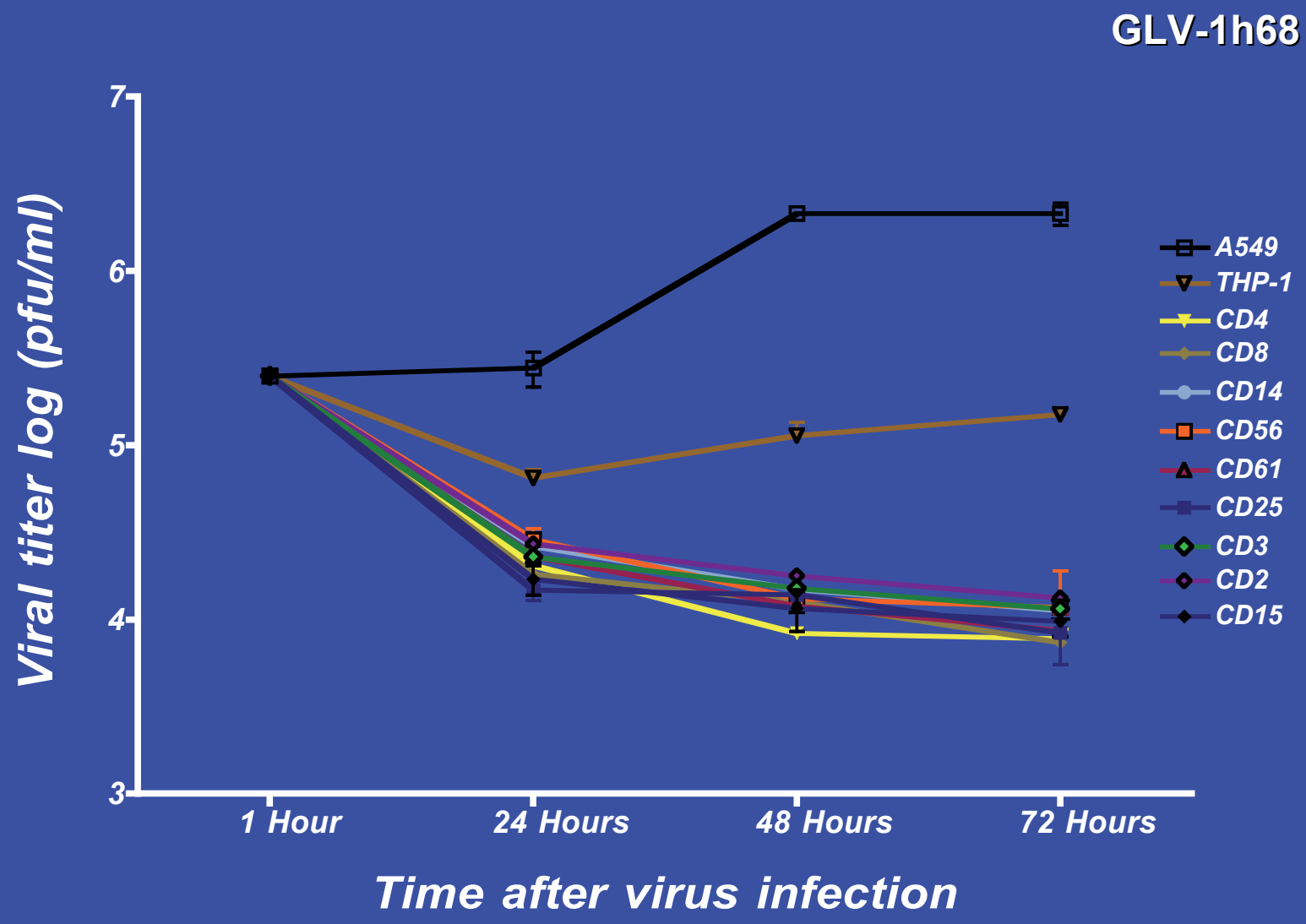
GLV-0b347; MOI:1



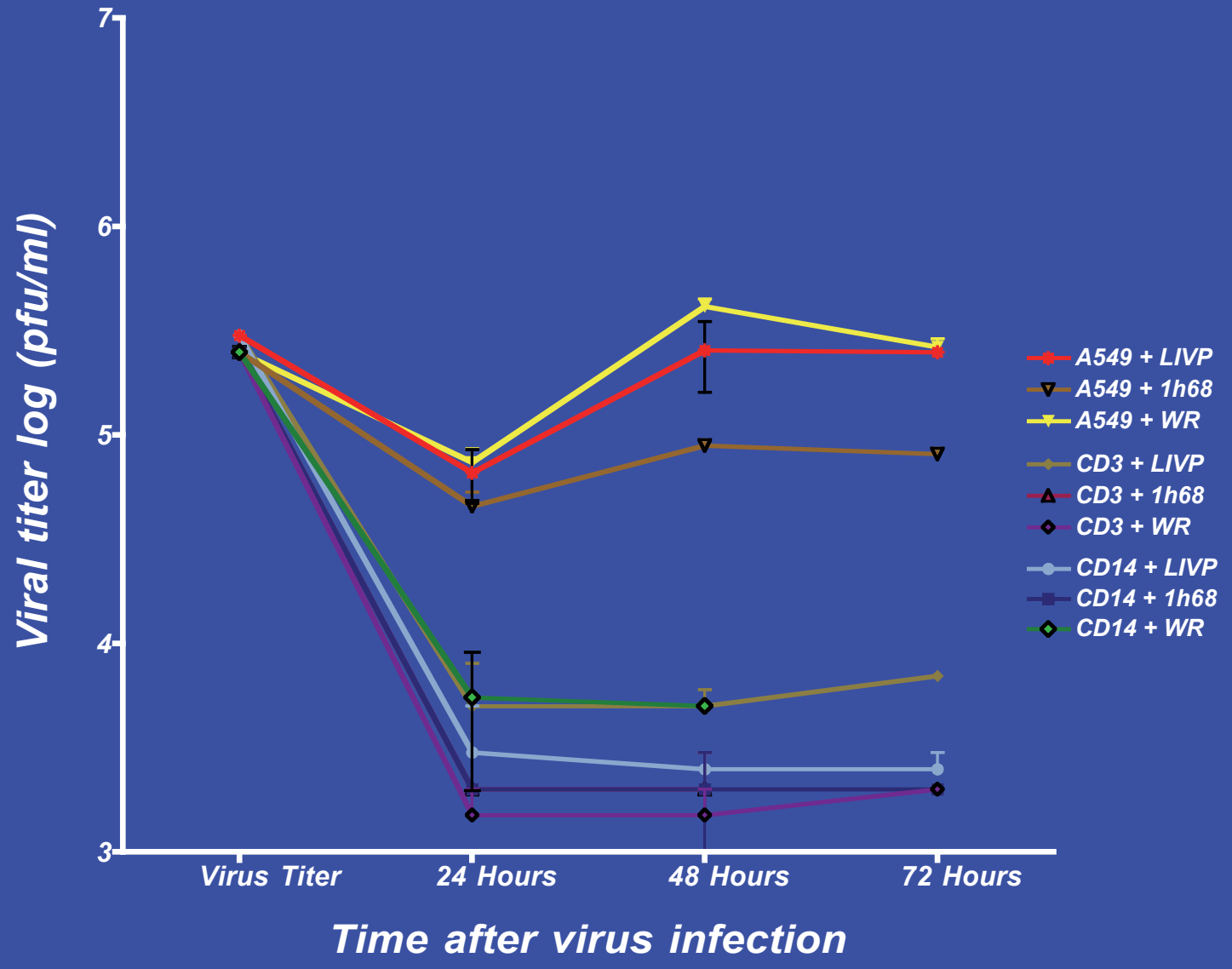
VACV Infection of Dendritic Cells



VACV Replication in PBMC Subsets



VACV Replication in PBMC Subsets



Conclusions

- All tested oncolytic virus constructs preferentially infect monocytes and activated T cells, followed by B-lymphocytes and NK cells (under optimal *in vitro* conditions). In contrast, the resting T lymphocytes were weakly infected.
- The LIVP 1.1.1.-based construct (GLV-2b372) was the most efficient in infecting the mononuclear cell subsets, followed by the WR-based construct (GLV-0b347).
- Cytotoxicity of all virus constructs for the infected mononuclear cells was similar at each tested time point.
- The results of the plaque assays for viral replication suggested an abortive infection in all tested subsets including activated T cells.
- In all experiments, the viral amplification and cytotoxicity were significantly higher in the control cancer cells than in any of the mononuclear cell subsets.

Chronic MyeloMonocytic Leukemia (CMML)

- **Definition**

  Malignant neoplasm exhibiting both myelodysplastic and myeloproliferative features and characterized by peripheral monocytosis

- **Diagnostic Criteria**

  1. Persistent peripheral blood monocytosis >1 $x10^9$/L
  2. No Philadelphia chromosome or BCR-ABL1 fusion gene
  3. No rearrangement of PDGFRA or PDGFRB (should be specifically excluded in cases with eosinophilia)
  4. <20% blasts (including myeloblasts, monoblasts and promonocytes) in the blood and bone marrow
  5. Dysplasia in one or more myeloid lineages. If myelodysplasia absent or minimal, diagnosis can still be made if requirements 1-4 are met, and:
     an acquired clonal cytogenetic or molecular genetic abnormality is present, or
     the monocytosis has persisted for at least 3 months, and
     all other causes of monocytosis have been excluded (e.g. infection, inflammation, malignancy)

Chronic MyeloMonocytic Leukemia (CMML)

- **Prognostic groups**

CMML-1

Blasts + promonocytes <5% in blood and <10% in marrow

CMML-2

Blasts + promonocytes 5-19% in blood and 10-19% in marrow OR
Auer rods present in blood or marrow

- **Phenotype of CMML Cells**

MONOCYTIC CELLS IN CMML:

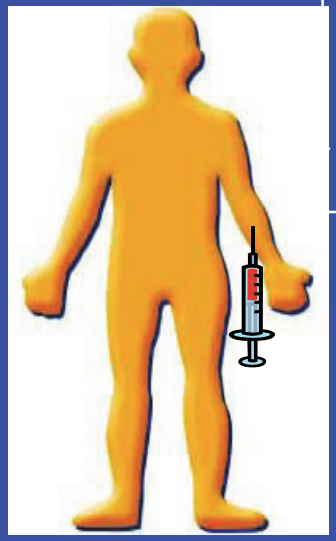
- Partial loss of CD13, CD14, and CD15
- Variable expression of CD56 and lack of expression of CD34 and CD117
- Expression of CD33, CD11b, and CD64

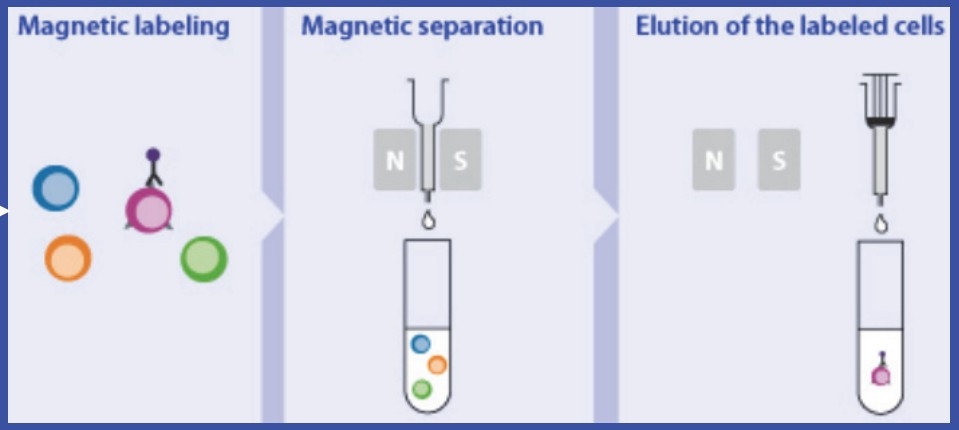
MONOBLASTS IN CMML:

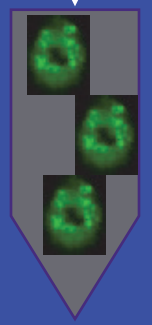
- Expression of CD34 and CD117
- Loss of expression of CD11b, CD14, CD64, and CD15

EXPERIMENTAL PROCEDURES

RBC lysis, virus infection, cytospin, microscopy





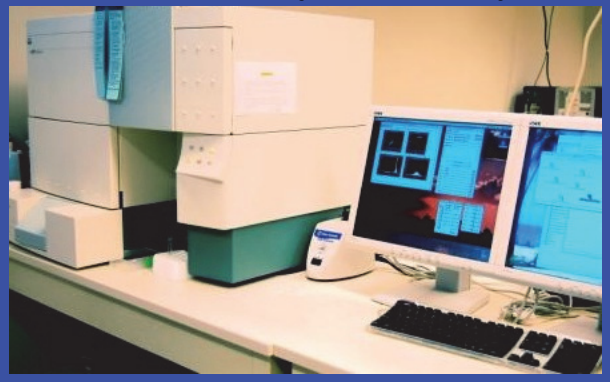


Virus-infected cells

Magnetic bead separation, infection

Healthy donor and Patient

Flow Cytometry



Fluorescent Microscopy

Virus Infection Experiments

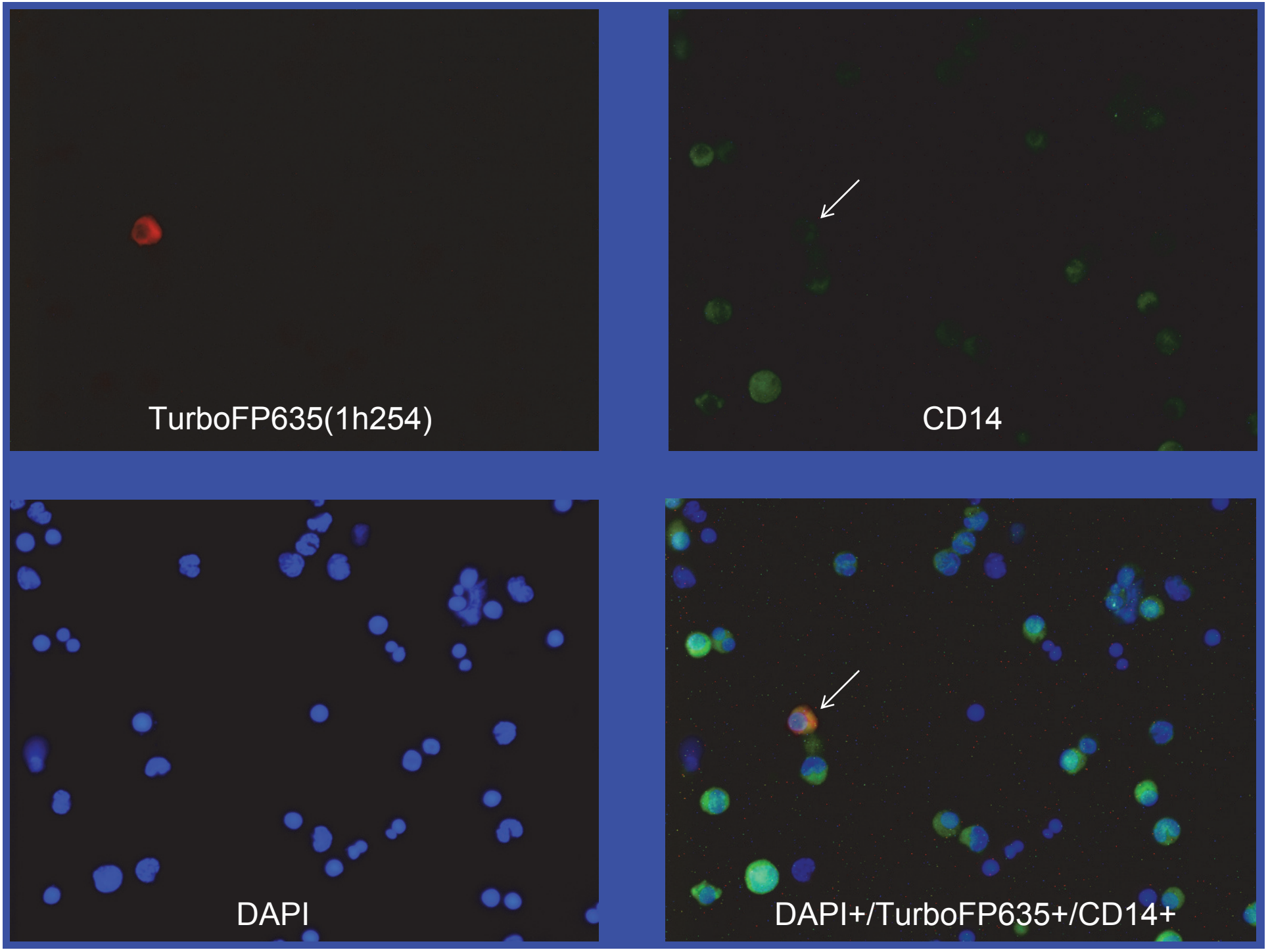
Viruses Used

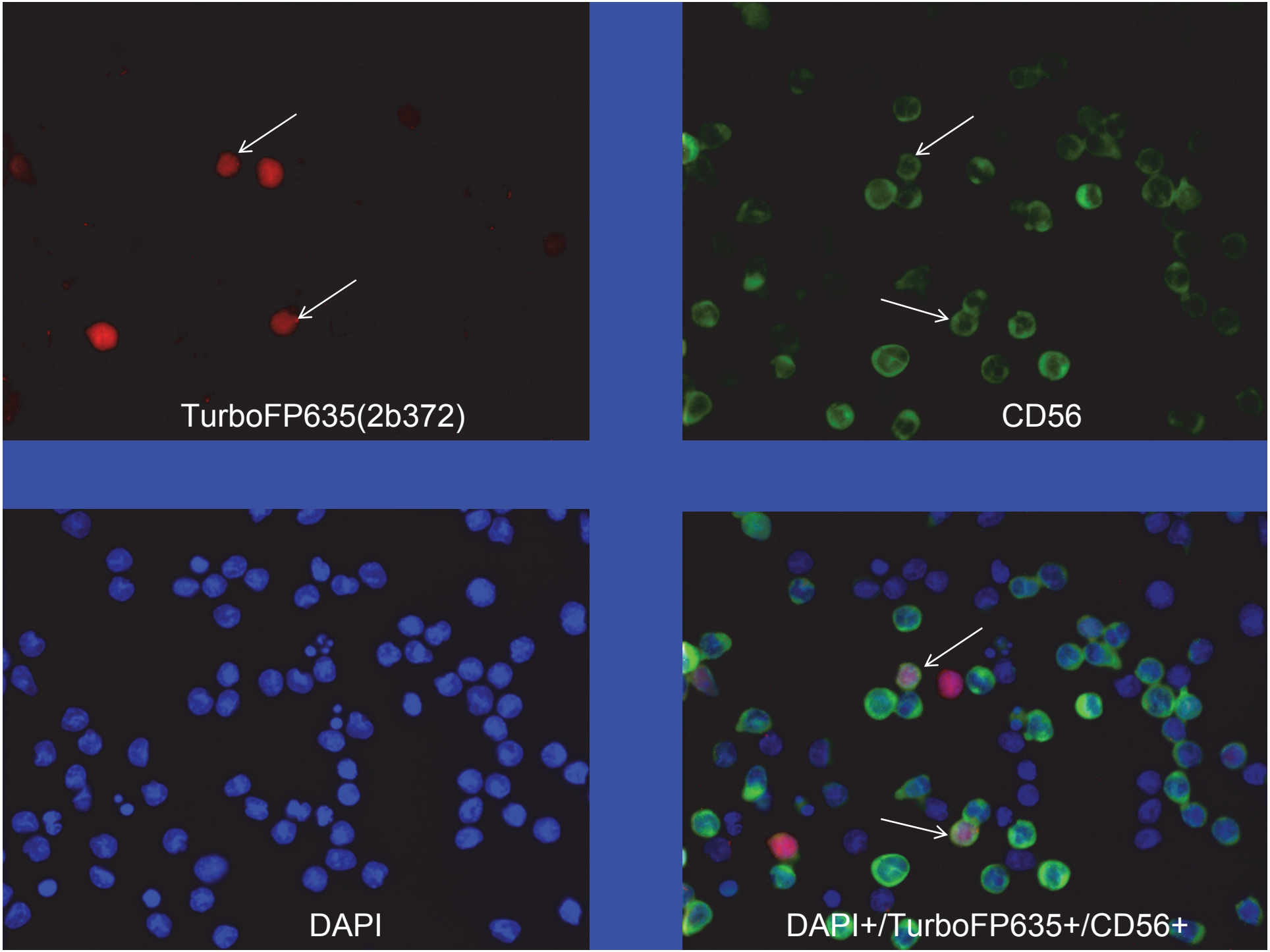
- **GLV-1h254**
  - Backbone: LIVP
  - Insert: TurboFP635
  - MOI 1
  - Incubation for 24 Hours at 37ºC

- **GLV-2b372**
  - Backbone: LIVP 111
  - Insert: TurboFP635
  - MOI 1
  - Incubation for 24 Hours at 37ºC

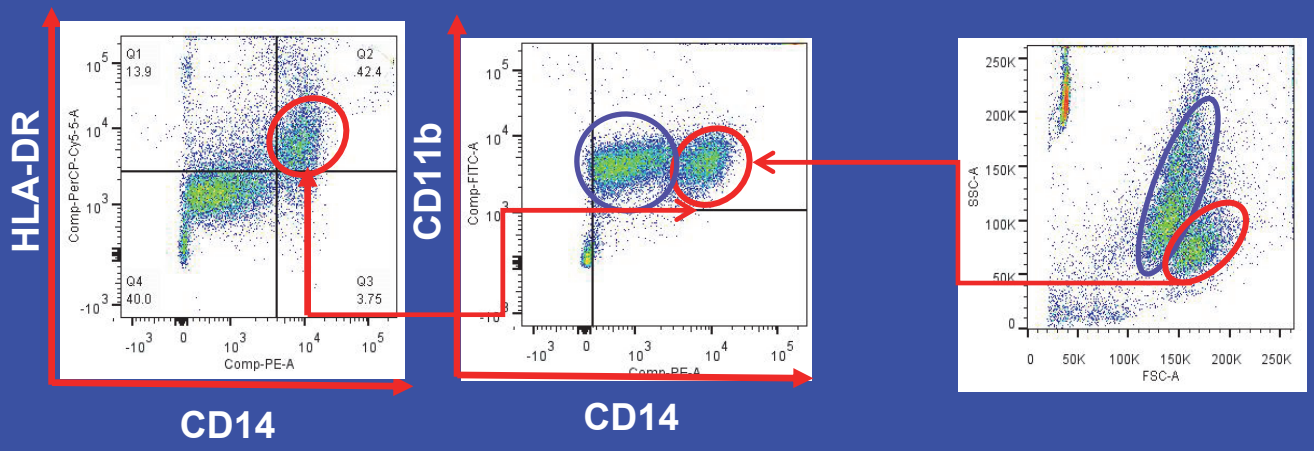
Virus Infection Experiments (Numbers of Isolated Cells)

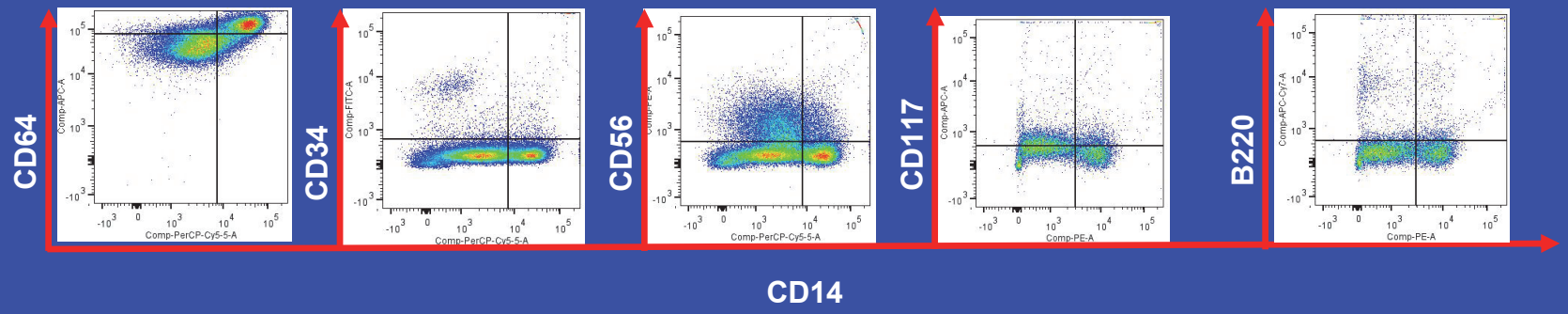
CELLS AND VIRUSES	HEALTHY DODOR	CMML PATIENT
PBMC Isolated from 13ml of blood	$30x10^6$	$560x10^6$
Isolated CD14+ cells	$0.13x10^6$	$23x10^6$
Isolated CD19+ cells	$0.62x10^6$	$0.9x10^6$
Isolated CD56+ cells	$0.28x10^6$	$29.3x10^6$
Isolated CD66+ cells	$2x10^6$	$66x10^6$
MOI of 1h254 and 2b372 viruses	MOI 1	MOI 1


TurboFP635(1h254)
CD14
DAPI
DAPI+/TurboFP635+/CD14+


TurboFP635(2b372)
CD56
DAPI
DAPI+/TurboFP635+/CD56+

Phenotype of CD14+ Monocytes Isolated from Patient's PBMC

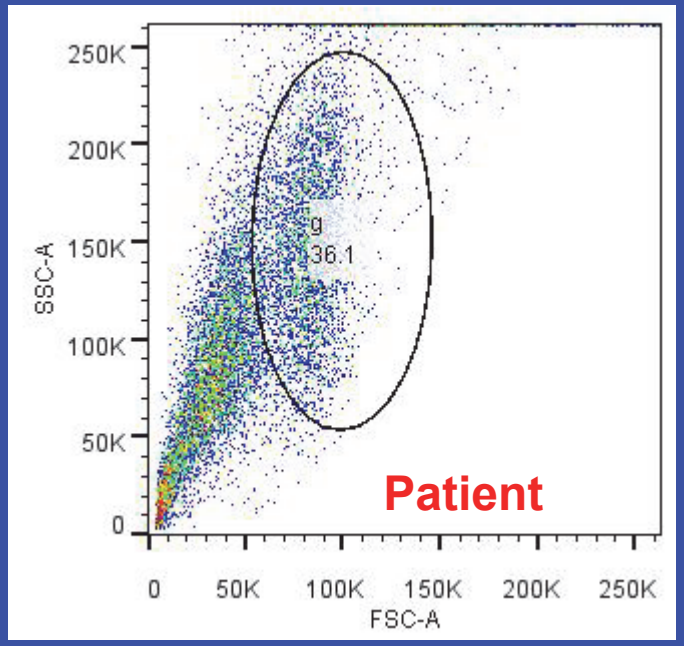


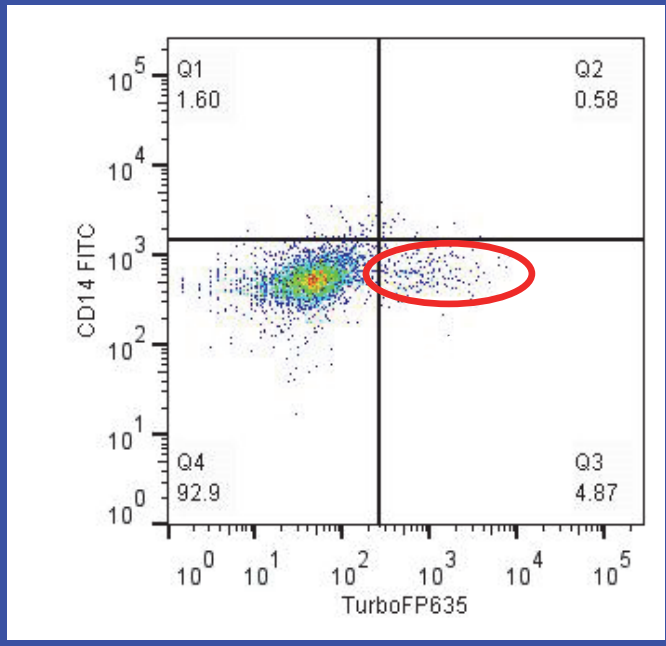


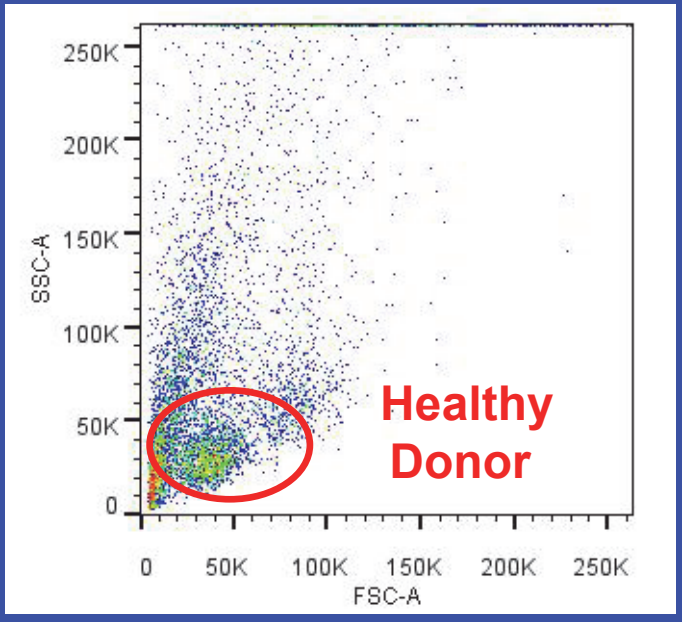
Phenotype:

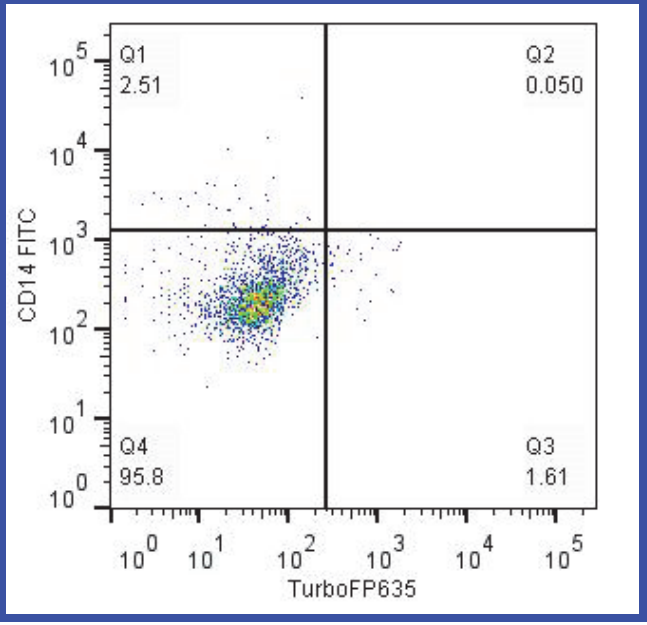
CD14 low, CD56+/-, HLA DR-, CD34-, CD11b +, CD117low, B220-, CD64low

Infection of Monocytes with GLV-2b372

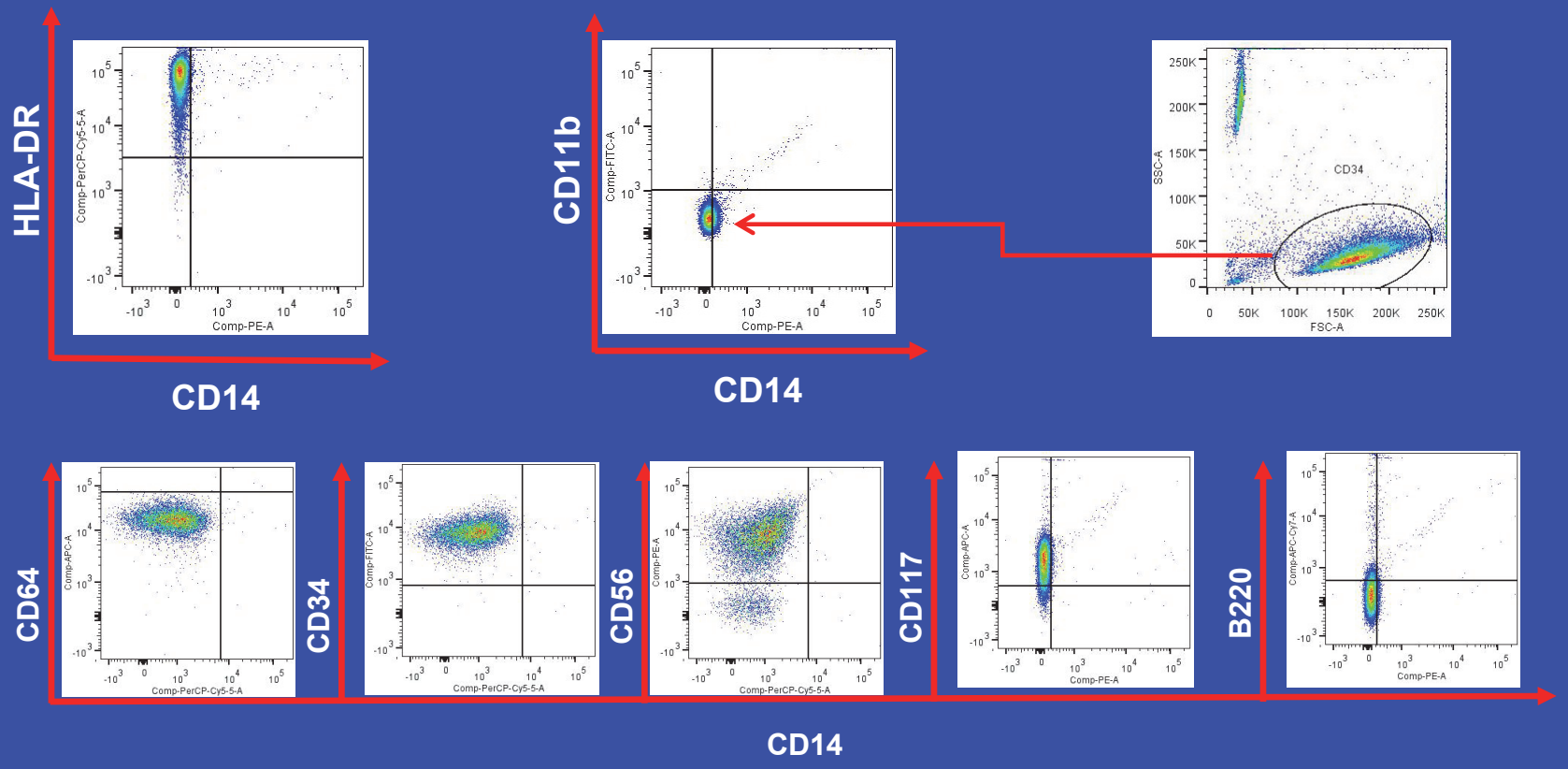








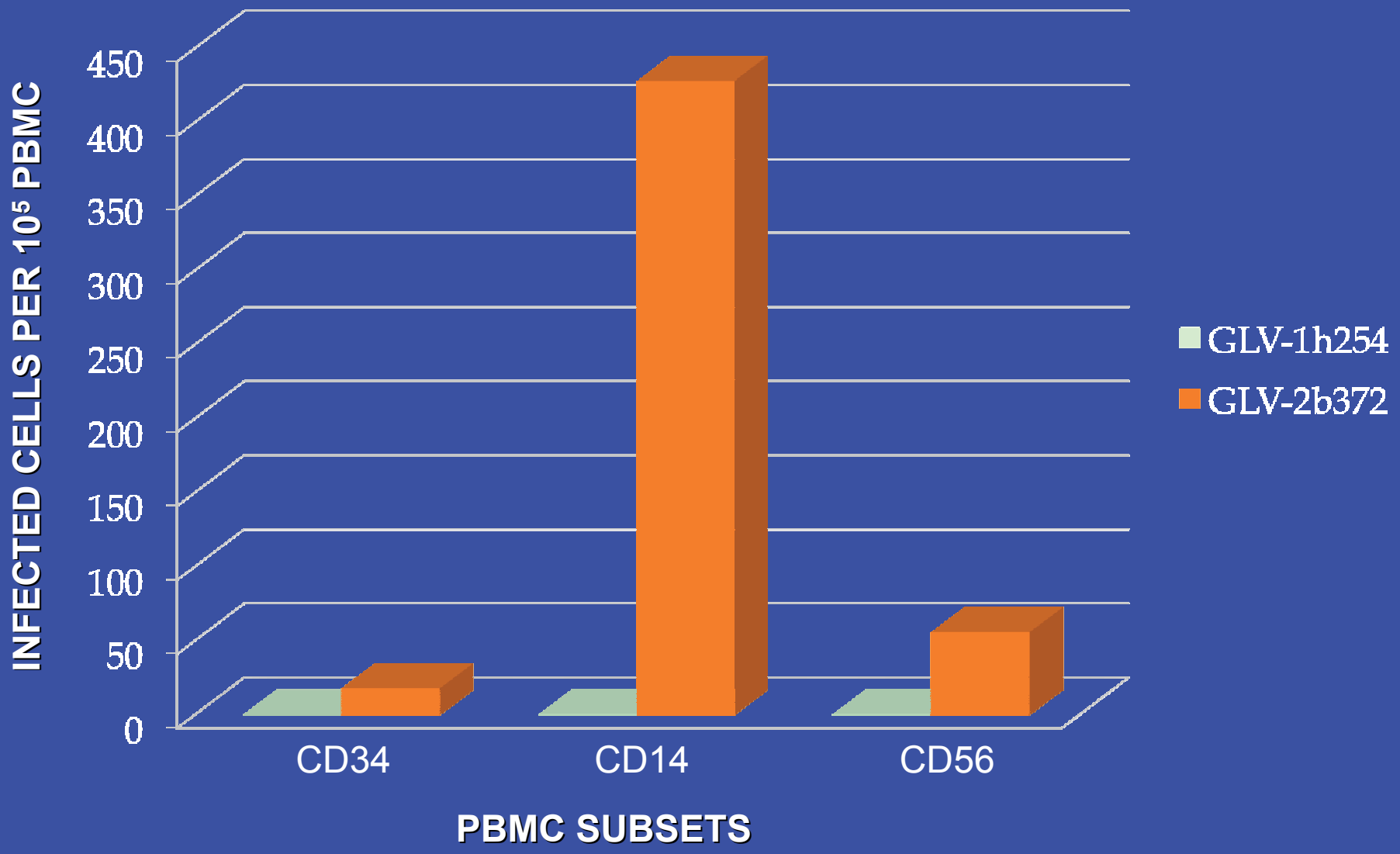
Phenotype of CD34+ Cells Isolated from Patient's PBMC



Phenotype:

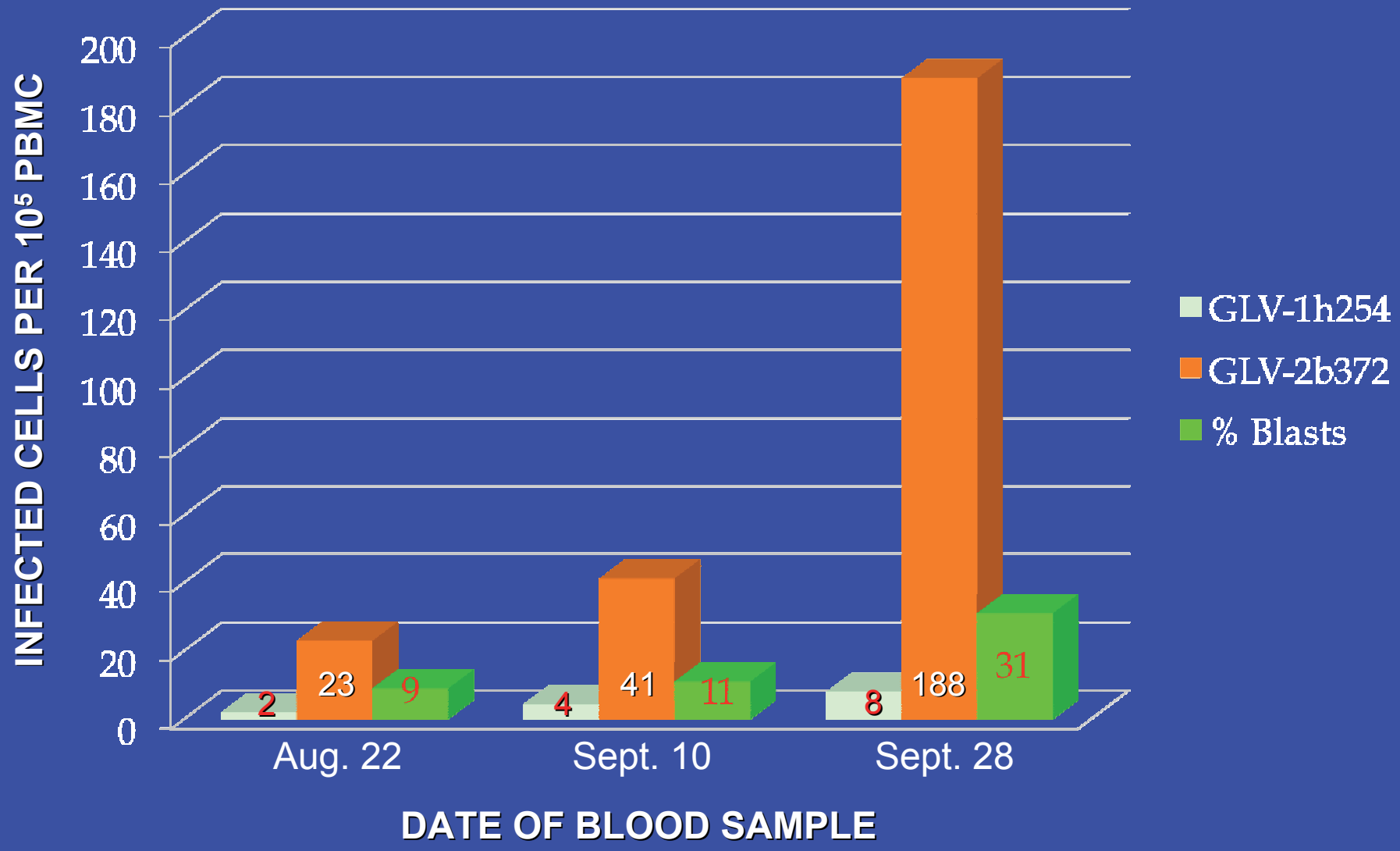
CD34+, CD117 +, CD11b-, CD14-, HLA DR+, B220-, CD56+, CD64low

VACV-Infected PBMC Subsets



Virus Infection Correlates with Disease Progression

VACV-Infected PBMC



Conclusions

- Virus infection with both viruses (GLV-1h254 and GLV-2b372) correlates with the disease progression in this patient with CMML
- Virus GLV-2b372 can infect CMML patient-derived blood subsets CD14, CD56 and CD34 (Blasts)
- Virus GLV-1h254 can infect CMML patient-derived total WBC and the blood subset CD56, although much less efficiently than the virus GLV-2b372
- Flow cytometry data suggest that the infected cells have blast-like characteristics

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