BioResearch

Efficient Transfection of Biologically Relevant Cells in Immunology Research

13 May 2014 / Speaker: Sean Fuerst
14 May 2014 / Speaker: Dr. Isabella Drewelus

Lonza

Agenda

- Technology Introduction
- Nucleofection™ of Immune Cells – Application Examples
  - Generation of specific antigen-presenting cells for stimulating cytotoxic T lymphocytes
  - Suitability of Nucleofection™ for Recombinant T Cell Receptor Transfer
- Tips and Tricks for Successful Nucleofection™
- Nucleofection™ Portfolio – Serving Different Needs
- Summary

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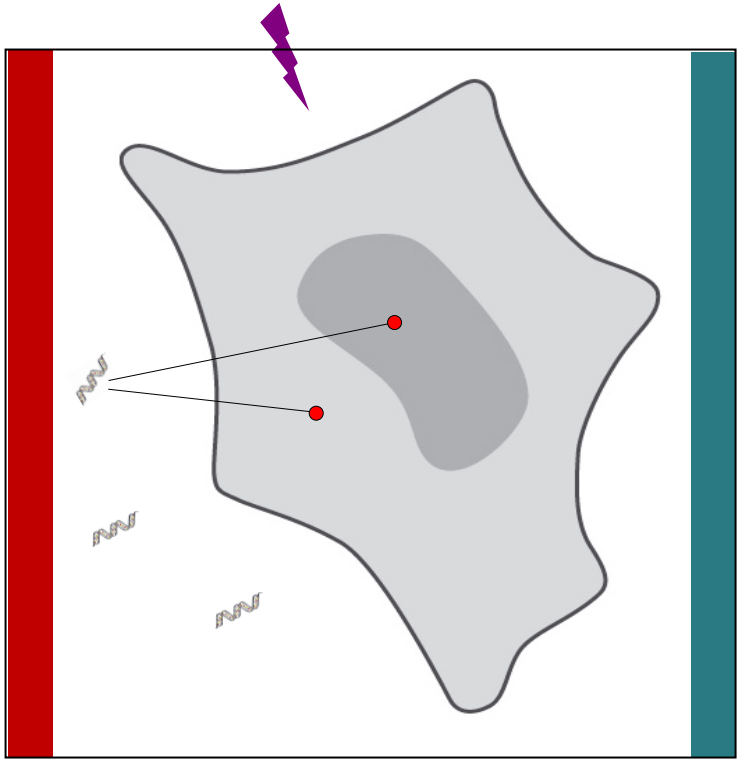
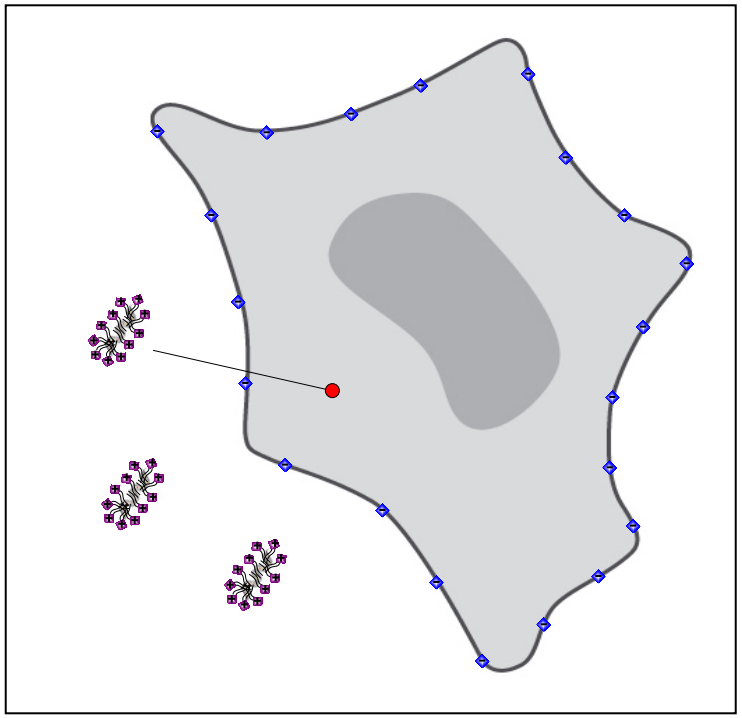
Nucleofection™ – The Principle

- High transfection efficiency combined with low mortality
- DNA is directed into the nucleus giving faster gene expression



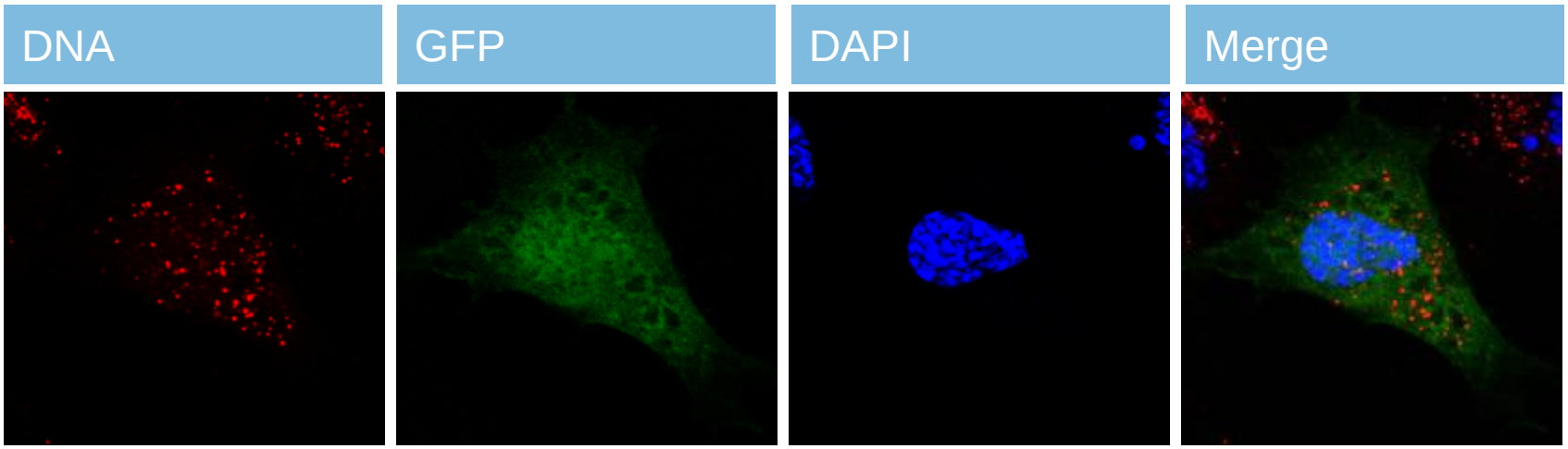
Nucleofection™



Other transfection methods

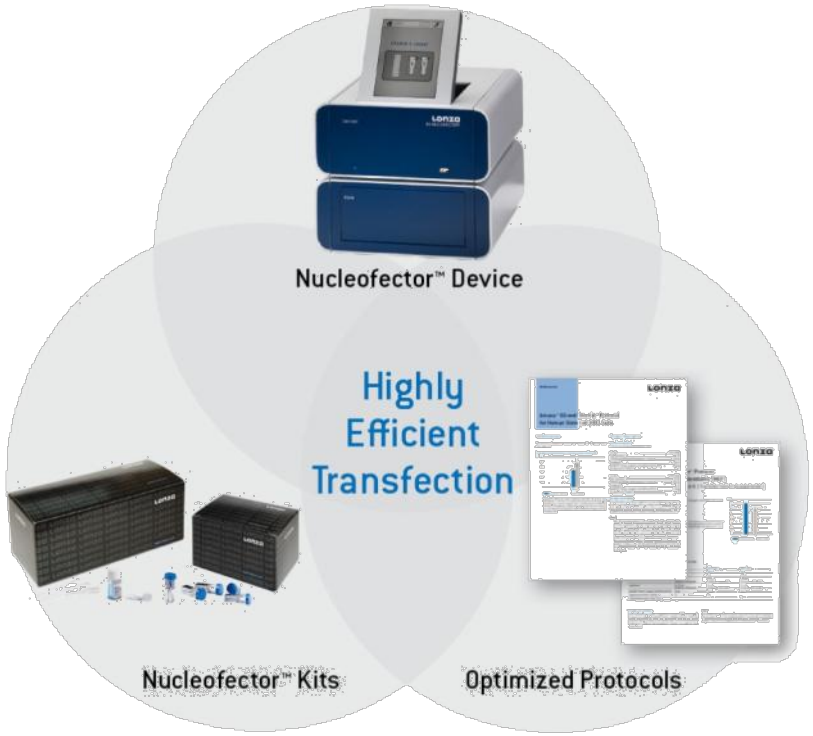
Proven Transport of DNA Into the Nucleus

Transport | Expression | Nucleus



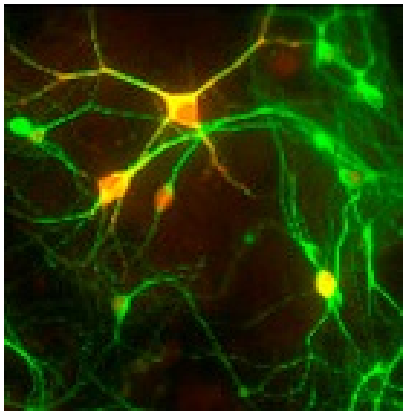
- Primary NHDF-neo cells were transfected with labeled plasmid DNA encoding GFP, fixed after 2h in 3.5% PFA and analyzed by confocal microscopy.

Components of Nucleofector™ Technology



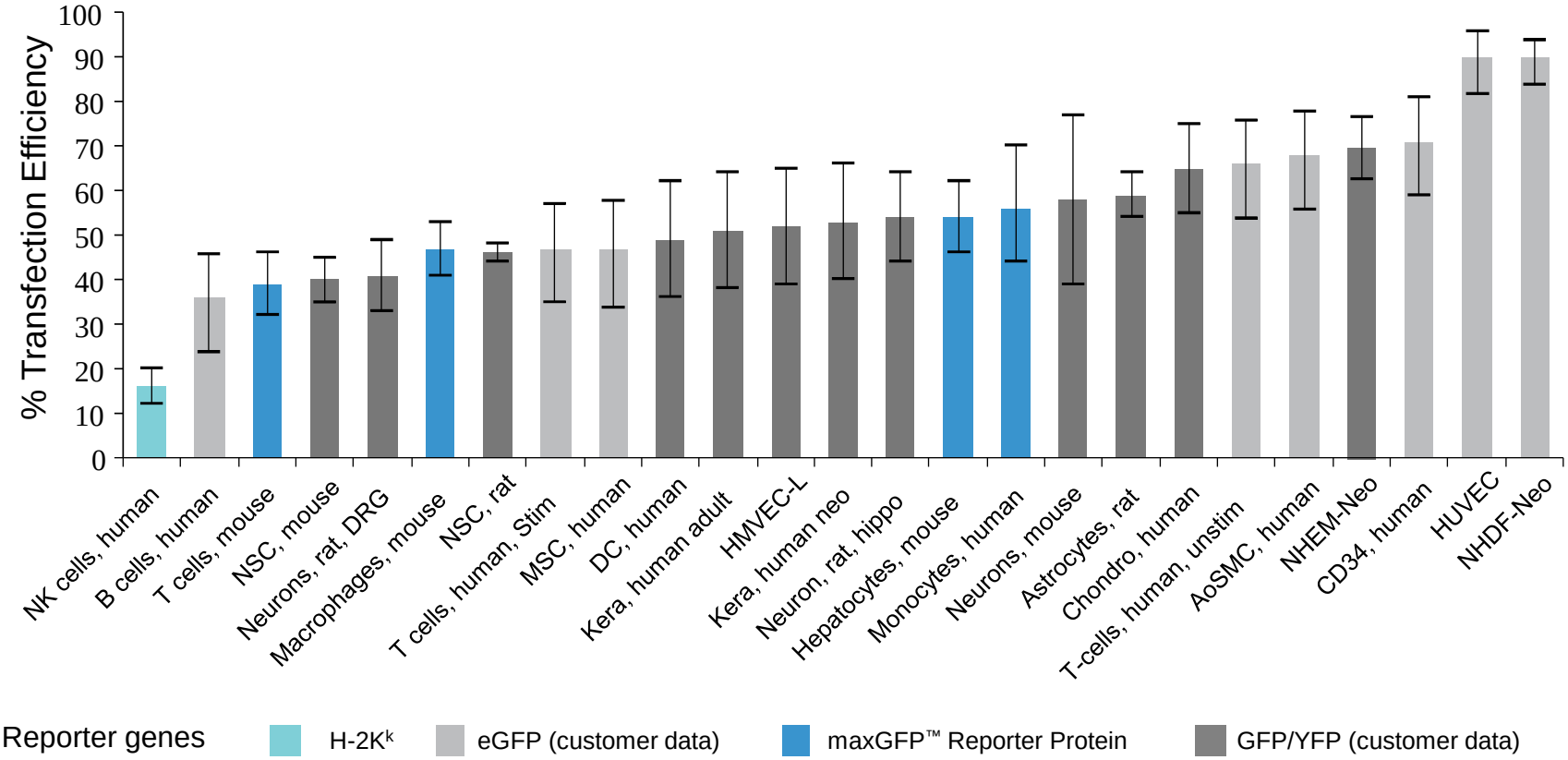
A Unique Combination:

- Nucleofector™ Device
- Specific Nucleofector™ Kits
- Detailed optimized protocols
- Enabling excellent transfection performance combined with high functionality!



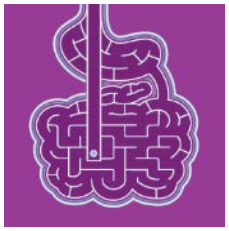
More Than 80 Primary Cell Types Successfully Transfected

- Analysis time: 16-48 h post Nucleofection™

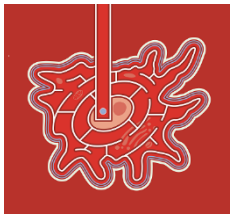


Nucleofection™ Applications Overview

- Immunology
  - Human T cells
  - Immune system cells

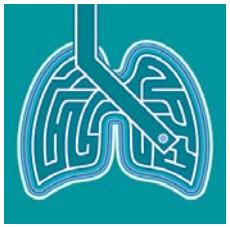


- Metabolic research
  - Chondrocytes
  - INS-1, MIN-6
  - Adipocytes, 3T3-L1



- Cancer
  - Mammary epithelial cells
  - Prostate epithelial cells

- Neurobiology
  - Cortical neurons
  - Dorsal root ganglia

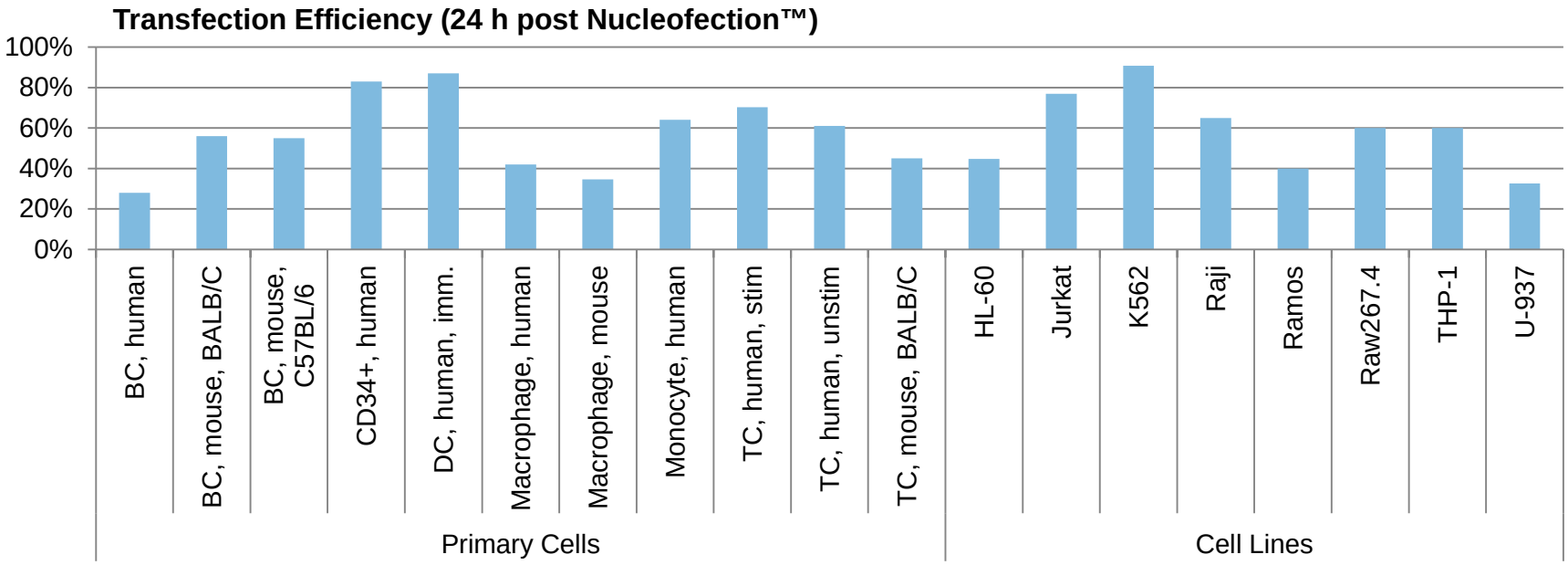


- Respiratory/ Cardiovascular research
  - Bronchial epithelial cells
  - Cardiomyocytes
  - HUVEC, HMVEC, SMC

- Stem cell research
  - Human fibroblasts
  - Human CD34+ cells
  - Human monocytes

More Than 45 Blood Cell Types Successfully Transfected

- Just a few examples…



Nucleofection™ Applications in Immunology Research

- HIV research, e.g.
  - Study of mechanisms that lead to apoptosis of CD4+ T cells upon HIV infection (Laforge M et al., 2007)

- T-cell differentiation, e.g.
  - Identification of genes involved in Th1 and Th2 cell differentiation (Lund RJ et al., 2007). Th subtypes are involved in the pathogenesis or progression of many immune-mediated diseases, such as type 1 diabetes and asthma
- Regulation of cytokine expression or cytokine signaling pathways (e.g. via reporter gene assays)
- Generation of specific antigen-presenting cells (APCs, e.g. dendritic cells or B cells) for subsequently stimulating cytotoxic T lymphocytes (CTLs)

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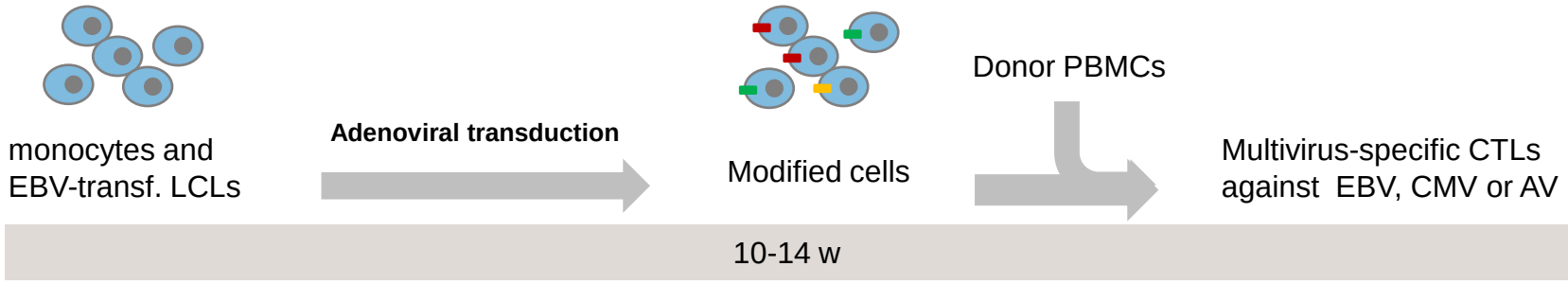
Treatment or Prevention of Viral Infections with Multivirus-Specific T Cells (1)

Gerdemann U., Vera J.F., Rooney C.M., Leen A.M. (2011).
Generation of Multivirus-specific T Cells to Prevent/treat Viral Infections after Allogeneic Hematopoietic Stem Cell Transplant. JoVE. 51. http://www.jove.com/details.php?id=2736

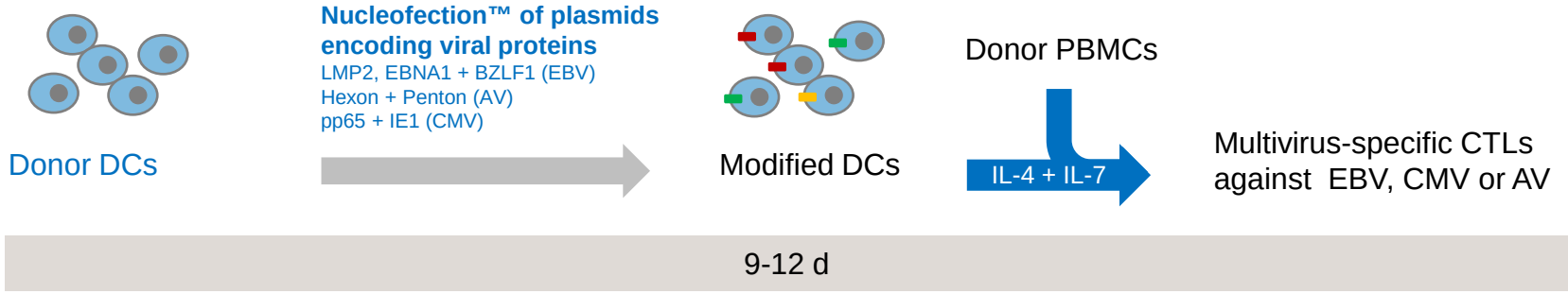
- Background
  - Viral infections can be severe side effects for transplantation of allogeneic hematopoietic stem cells due to immuno-suppression of recipients
  - Infusion of virus-specific cytotoxic T lymphocyte (CTL) from the stem cell donor have proven effective for prevention or treatment

Treatment or Prevention of Viral Infections with Multivirus-Specific T Cells (2)

- Approach so far:

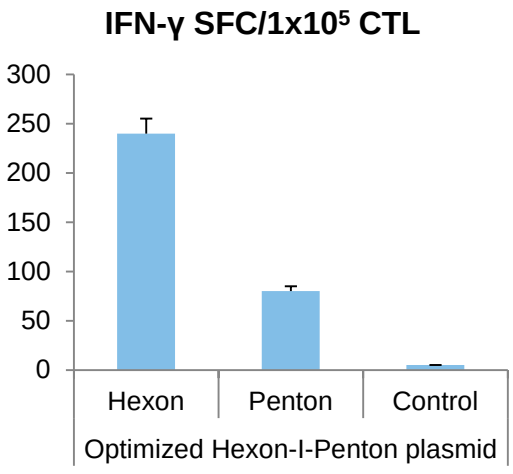


- New approach:

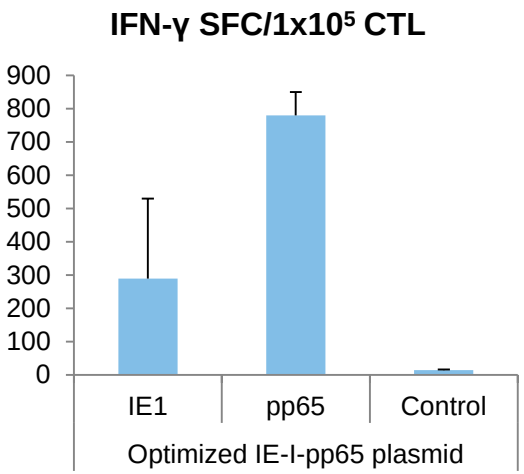


Treatment or Prevention of Viral Infections with Multivirus-Specific T Cells (3)

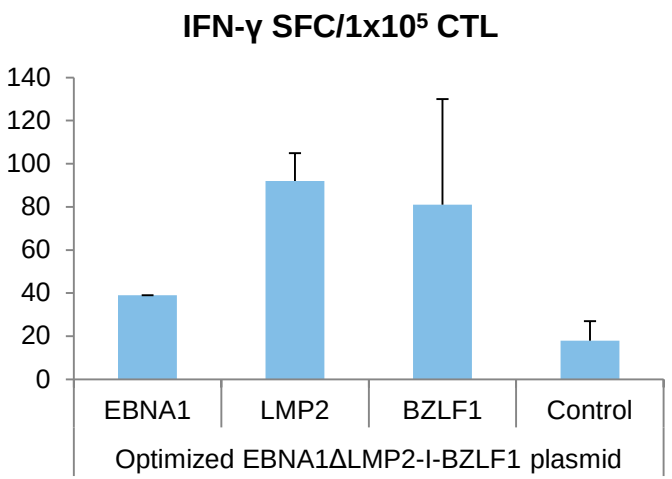
- Transfected human DCs were used to stimulate T cells and specificity was analyzed by IFNγ ELIspot 10 days post-stimulation



AV-specific CTLs



CMV-specific CTLs



EBV-specific CTLs

Data reproduced. with permission of Ann Leen, NIH

Treatment or Prevention of Viral Infections with Multivirus-Specific T Cells (4)

- Results:
  - Due to non-viral transfection there is no competition of specific antigens of interest with other viral antigens
  - Optimized CTL culture conditions improved cell survival and proliferation
  - Protocol enables generation of CTLs with the correct phenotype and functionality (data not shown here)
  - Significant time savings (~2 weeks vs 10-14 weeks)
  - Simple protocol, FDA approved

- Conclusion:
  - Reduction of time, cost and complexity may allow broader implementation of T-cell immunotherapy
  - Approach can be easily extended to other viruses provided that protective antigens are identified

Data with permission of Ann Leen, NIH

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Suitability of Nucleofection™ for Recombinant T Cell Receptor Transfer

Field AC, Vink C, Gabriel R, Al-Subki R, Schmidt M, et al. (2013)
Comparison of Lentiviral and Sleeping Beauty Mediated αβ T Cell Receptor Gene Transfer. PLoS ONE 8(6): e68201. doi:10.1371/journal.pone.0068201

- Background:
  - Transfer of recombinant T cell receptors, e.g. chimeric antigen receptors (CARs), into T cells → redirecting T cell immunity against tumor or viral antigens

Retro- or lentiviral transduction
- High effort: New viruses required when testing different receptor constructs
- High-costs: Manufacturing clinical grade viral vectors

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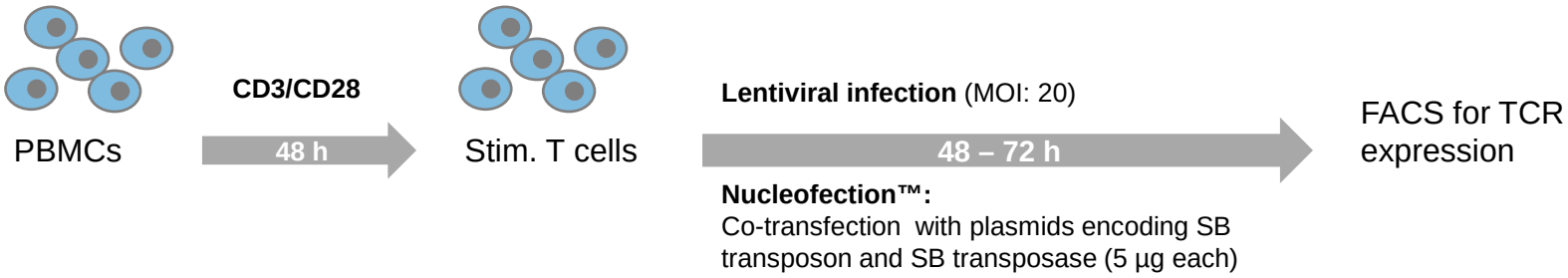
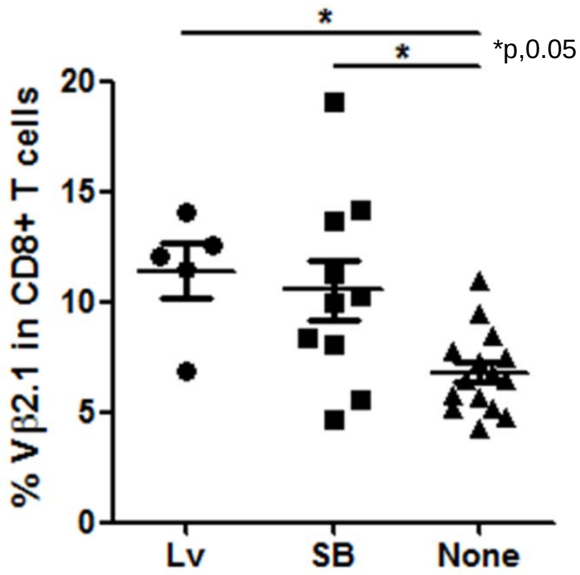
Plasmid-based gene transfer
using transposable elements, e.g. Sleeping Beauty (SB)

- This study:
  - Comparison of lentiviral transduction (LV) versus Nucleofection™ of an enhanced Sleeping Beauty transposition system (SB100X, a hyperactive SB transposase) to deliver a codon optimized αβ TCR against WT1 antigen

Comparable TCR Expression Levels



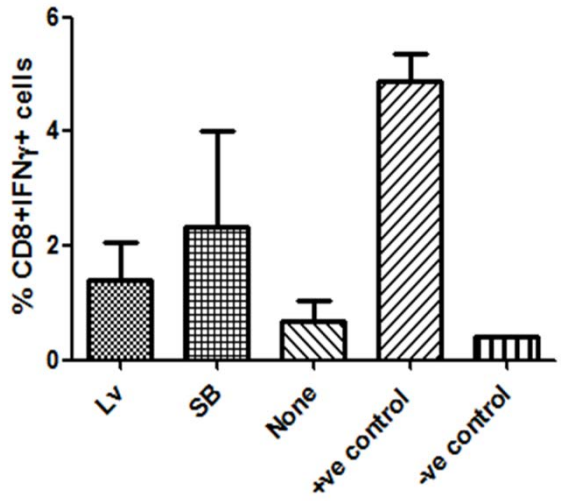


- Results: Comparable levels of WT1-TCR expression for lentiviral versus SB-mediated transfer into CD8+ T cells (donor variability)
  - Lentiviral transduction: 11.4% (n =5)
  - SB mediated: 10.5% (n =10)

From: Field A-C, Vink C, Gabriel R, Al-Subki R, Schmidt M, et al. (2013) PLoS ONE 8(6): e68201. doi:10.1371/journal.pone.0068201,. With premission.

Comparable In Vitro Functionality

- IFNγ release of modified CD8+ T cells after exposure to
  - a WT1 peptide
  - anti-CD3 Ab (positive control)
  - irrelevant peptide (negative control)



- Results:
  - LV transduction: 1.4% of all $CD8^+$ $IFNc^+$ cells (= 28% of WT1 TCR-positive cells)
  - SB mediated: 2.3% of all $CD8^+$ $IFNc^+$ cells (= 57% of WT1 TCR-positive cells)

From: Field A-C, Vink C, Gabriel R, Al-Subki R, Schmidt M, et al. (2013) PLoS ONE 8(6): e68201. doi:10.1371/journal.pone.0068201,. With premission.

Conclusion

- Additional results (not shown here):
  - Confirmation of in vivo functionality of SB modified murine T cells in transgenic mice
  - Similar number of integration sites (n≥150) for both LV and SB, but integration within genes was higher with LV (70.8%) than with SB100X/transposon (40.2%)

- Conclusion: SB-mediated transfer of antigen-specific T cell receptor into T cells using Nucleofection™
  - Offers a flexible pathway for rapid evaluation of different receptor configurations
  - May allow – after further optimization and adaptation to GMP conditions – for therapeutic applications to be explored in early phase clinical studies

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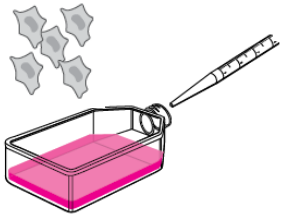
Things to Consider For Optimal Nucleofection™ Results

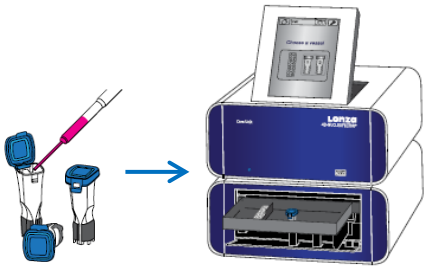
Low passage number
Log growth phase
No contamination
Donor differences
Minimize physical stress



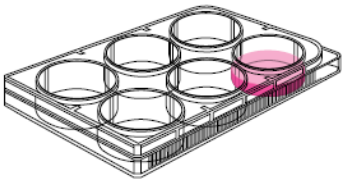
Highly purified, endotoxin-free
Low DNA amounts (or mRNA)

Equilibrate to RT
Minimize incubation with cells

Use optimized conditions



Fresh medium at 37 °C gentle seeding
No repeated aspiration
Wait at least 2-4 h before adding stimulants



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Nucleofection™ Portfolio Tailored to Your Needs

					
Device	**4D-Nucleofector™ X Unit**	**4D-Nucleofector™ Y Unit**	**96-Well Shuttle™ Add-on**	**HT Nucleofector™ System**	**Nucleofector™ 2b Device**
Throughput	Low (1-16)	Low (24)	Medium (96)	High (384)	Very low (1)
Format	Suspension	Adherent	Suspension	Suspension	Suspension
Reaction Volume	100 µl and 20 µl	350 µl	20 µl	20 µl	100 µl
Electrode Material	Conductive polymer	Conductive polymer	Conductive polymer	Conductive polymer	Aluminum
Cell Numbers	20 µl: 10^4 to 10^6 100 µl: 10^5 to 10^7	10^4 to 10^5	10^4 to 10^6	10^4 to 10^6	10^5 to 10^7

4D-Nucleofector™ System
Consumables Tailored for Your Application

100 µl Nucleocuvette™	20 µl Nucleocuvette™ Strip	24-well Dipping Electrode
		
10^5 - 10^6 cells	10^4 - 10^6 cells	
Suspension Nucleofection™		**Adherent Nucleofection™**

Benefit from

- Transfer of conditions from 20 µl to 100 µl
- Use of Conductive Polymer instead of metal electrodes
- Transfection cells in adherence

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Summary
Nucleofection™ of Immune Cells

Benefit from	
High efficiency combined with high viability/functionality	– Suited for notoriously hard-to-transfect cells, like primary blood cells or suspension cell lines – Up to 95% transfection efficiency with DNA or 99% with siRNA – No metal ion release with conductive polymer electrode platforms
High application flexibility	– Same conditions for various substrates (DNA, RNA, siRNA etc.) – Various platforms for different cell numbers or throughput
Ready-to-use, proven technology	– 40+ Optimized Protocols for blood/immune cells – 900+ immunology related publications

Your Lonza Immunology Tool Kit

Primary Cells and Media

- Poietics™ Primary Hematopoietic Cells (bone marrow, cord blood, CD34+)
- Chemically defined, serum-free media (e.g. X-VIVO™)
- BioWhittaker™ Classical Media (e.g. DMEM, RPMI)

Transfection

- Nucleofector™ Technology with optimized protocols for primary human or mouse cells, e.g.
  - T cells
  - B cells
  - Macrophages
  - Monocytes
  - CD34+ cells
  - Dendritic cells
- and numerous cell lines, e.g.
  - Jurkat
  - K562
  - THP-1
  - HL-60
  - U937
  - ……

Cell Analysis

- ViaLight™ or ToxiLight™ Cell Viability and Toxicity Assays
- MycoAlert™ Assay for mycoplasma detection in 20 min
- Precast gels for nucleic acid or protein electrophoresis

Sources of Information

Our Online Databases

- Citations: http://www.lonza.com/citations
- Cell Transfection: http://www.lonza.com/celldatabase
- Optimized Protocols: http://www.lonza.com/protocols

Transfection Experts

- Scientific Support Team EU: +32 87 321 611
  scientific.support.eu@lonza.com
- Scientific Support Team US: 800 521 0390 (toll free)
  scientific.support@lonza.com

Interested in Learning More?

- Join our upcoming webinar:

  Convenient Generation and Culture of Induced Pluripotent Stem Cells

  Slot 1: Tuesday, 24 June 2014
  2 PM EDT (New York) / 11 AM PDT (Los Angeles)

  Slot 2: Wednesday, 25 June2014
  10 AM CEST (Berlin) / 9 AM BST (London) / 5 PM JST (Tokyo)

- Register at: **www.lonza.com/webinar18**

Thank You for Your Kind Attention