# Development of an Optimized Lentiviral Transduction Medium and Process to Manufacture Genetically Modified MSC Working Cell Banks


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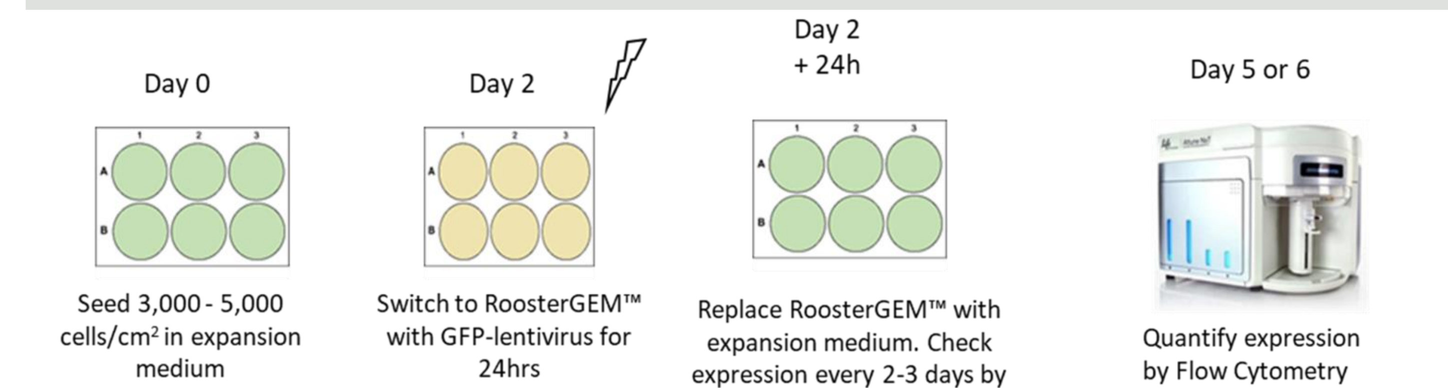
Radically Simplifying Use of MSCs


## ABSTRACT

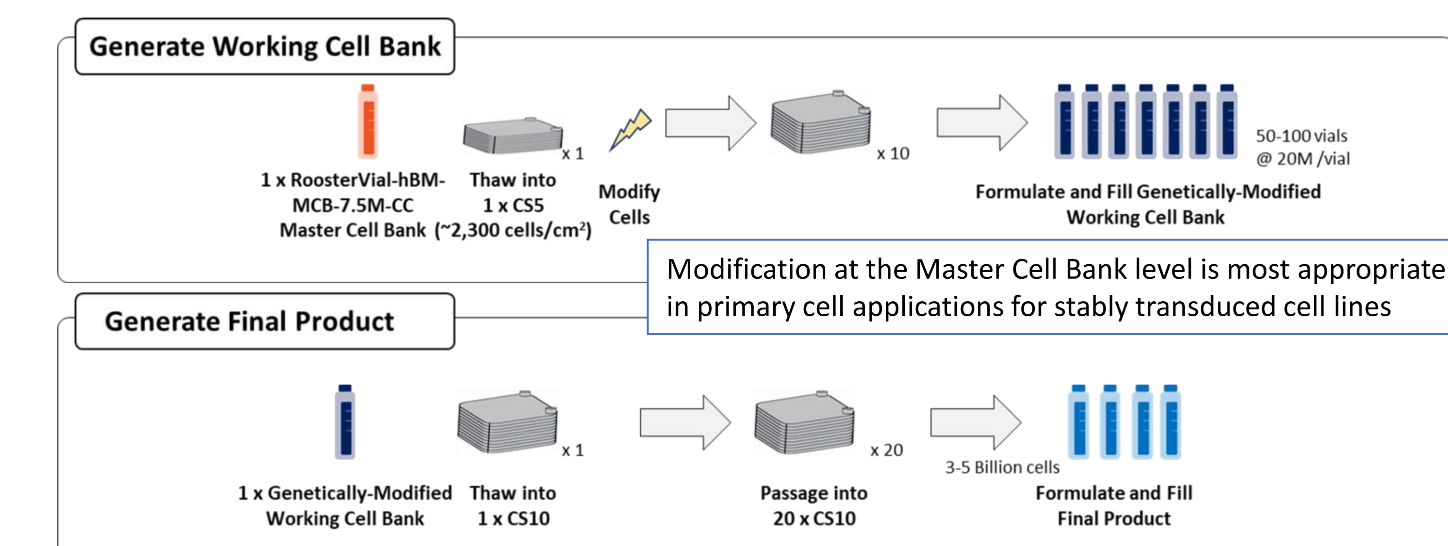
- There have been >1,000 clinical trials using human mesenchymal stem/stromal cells (hMSCs) for therapeutic applications, and their demonstrated safety profile make them ideal candidates for cell-based gene therapies.
- hMSCs may be genetically engineered to improve their inherent therapeutic properties or to produce different molecules for new applications.
- A critical challenge to engineered MSC therapeutic products is the historically low transduction efficiency of primary cells. Translating poor efficiency into cGMP manufacturing results in high costs, poor control, and inconsistent product quality.
- Our goal was to simplify the optimization of MSC genetic modification. We optimized the liquid reagents for maximum transduction efficiency and are offering it as an off-the-shelf medium for transduction/transfection. We then identified a small set of key process parameters to optimize that are project specific.
- We report here the development of a chemically defined, animal component free Genetic Engineering Medium (RoosterGEM™), and a robust process to manufacture scalable genetically modified hMSC Working Cell Bank (WCB) vials using lentiviral vectors.


## EXPERIMENTAL PROCESS FLOW



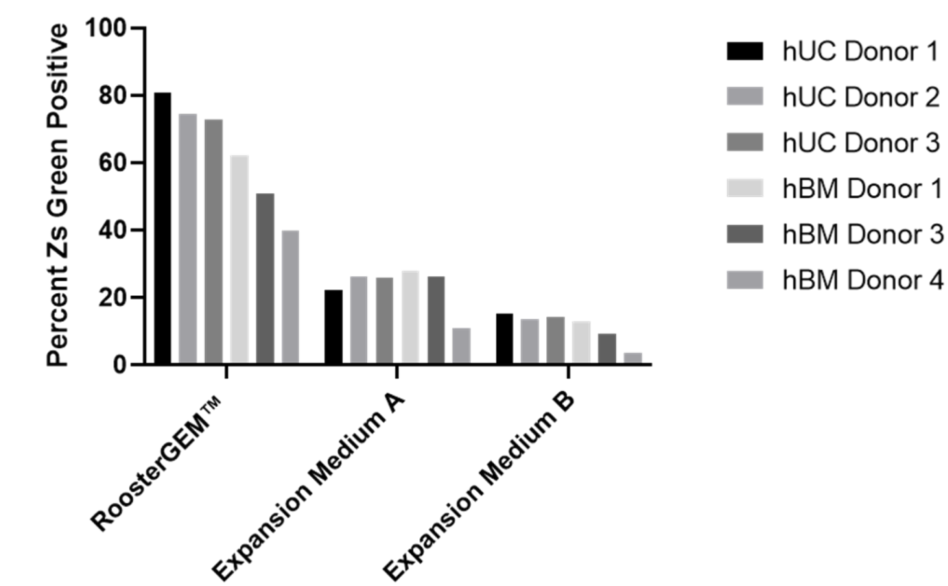

- hMSCs are seeded at 3-5000 cells/cm$^2$ on 6 well plates and grown for 2 days in expansion medium.
- Expansion medium is replaced with 1 ml/well RoosterGem™ containing lentiviral vector and incubated for 24 hours.
- RoosterGEM™ is replaced with 2 ml/well expansion medium.
- Expression is monitored by fluorescent microscopy every 2-3 days.
- On day 5-6, expression is quantified by Flow Cytometry.

## MANUFACTURING PROCESS FOR STABLY TRANSDUCED CELL BANKS

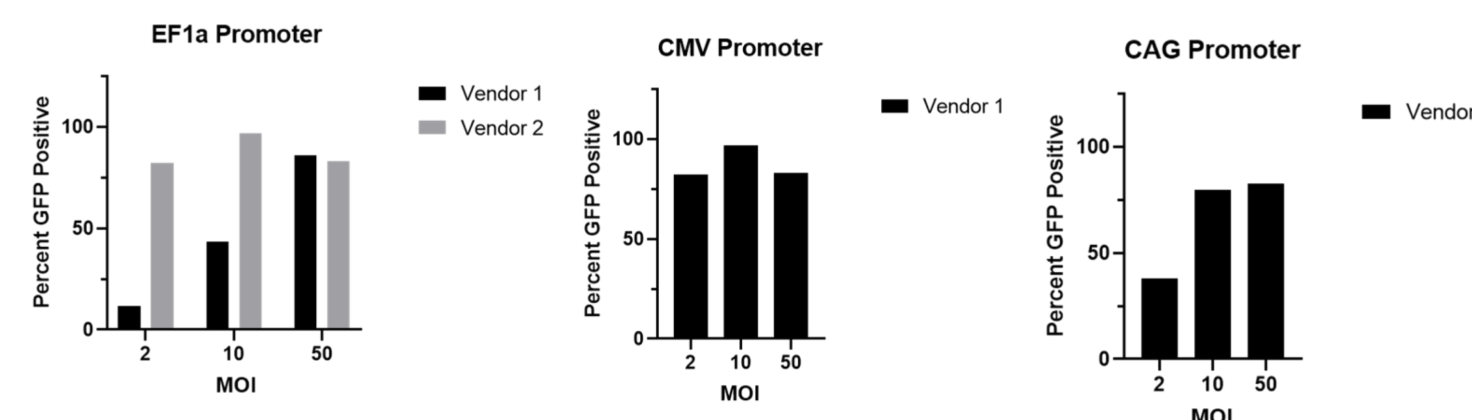



## CRITICAL PROCESS PARAMETERS (CPPs) FOR OPTIMAL LENTIVIRAL TRANSDUCTION INCLUDE MSC TISSUE SOURCE & DONOR, VIRUS MANUFACTURER & MOI; GENE PROMOTOR USED; AND VIRUS EXPOSURE METHOD

### Effect of Medium & Donor on Transduction Efficiency

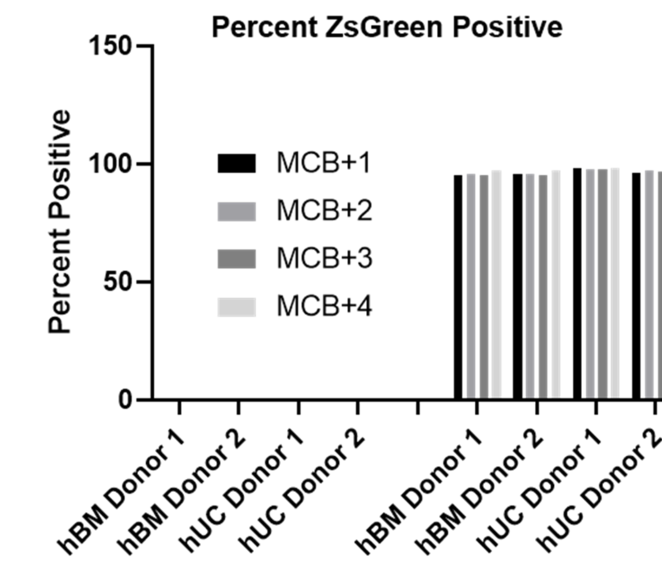


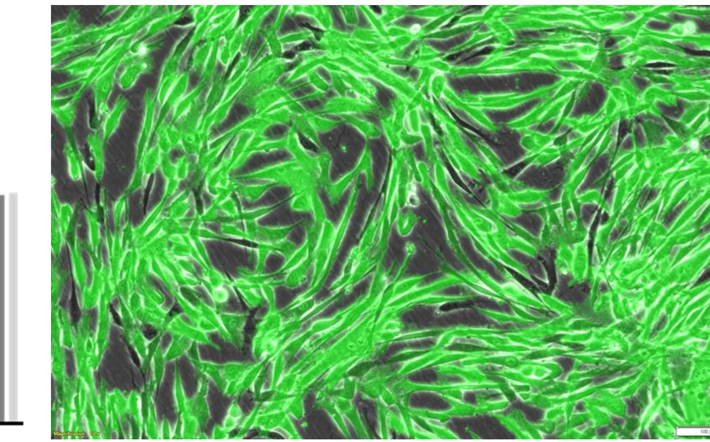

- The transduction efficiencies of 3 hUC-MSC and 3 hBM-MSC lots (MOI=2) were compared.
- hBM donors exhibited a range of efficiencies from 40% to 60%.
- hUC donors exhibited similarly high transduction efficiencies ranging from 70% to 80%.
- The use of RoosterGEM™ transduction medium resulted in the highest efficiencies across all donors tested.
- Spinoculation resulted in approximately 20% increased transduction efficiency in RoosterGEM™ and up to 80% increase in Expansion Media (Data not shown).



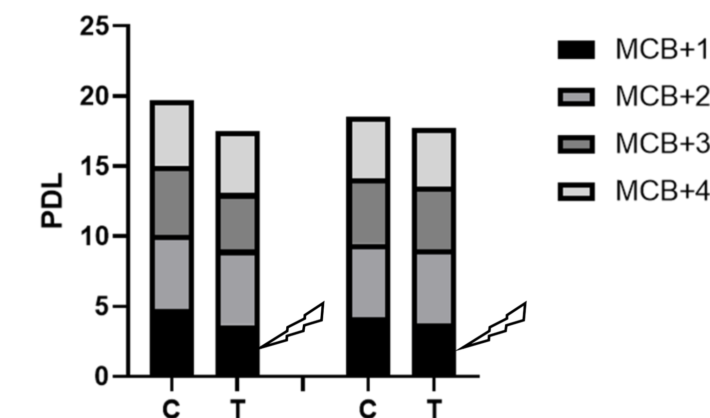

- The effect of lentivirus vendor and promotor were analyzed using hBM-MSC Donor 1.
- Virus containing the EF1a promotor from Vendor 2 resulted in higher transduction efficiencies at lower MOIs than that from Vendor 1.
- EF1a (Vendor 2) and CMV promotors supported similarly high transduction efficiencies, while CAG promoter produced best results at higher MOIs.
- Lentivirus containing EF1a promotor (Vendor 2) was used for the following experiments:

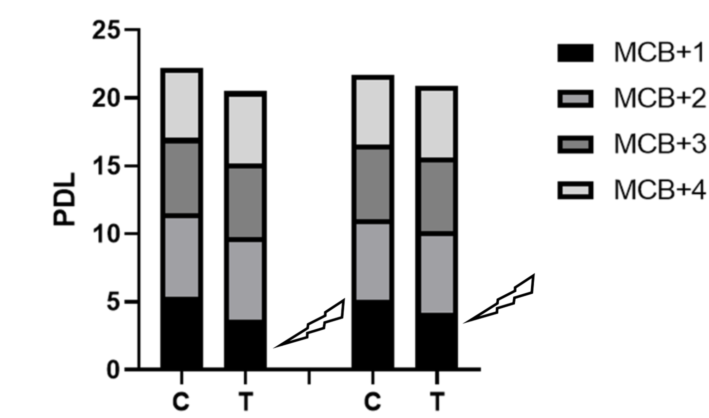
## LENTIVIRAL GENETIC MODIFICATION CAN BE IMPLEMENTED WITHIN AN MSC PRODUCTION PROCESS WITH MINOR IMPACTS ON CELL YIELDS AND COMPARABLE CQAS



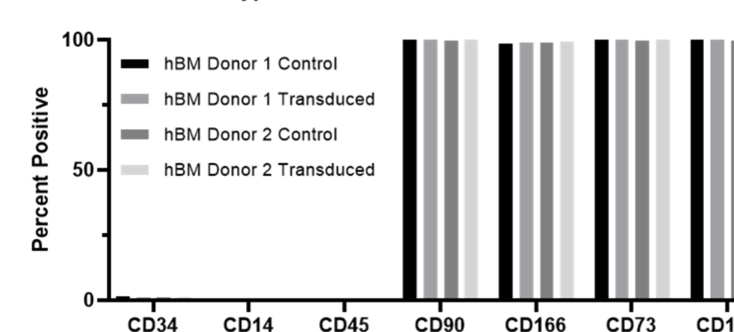



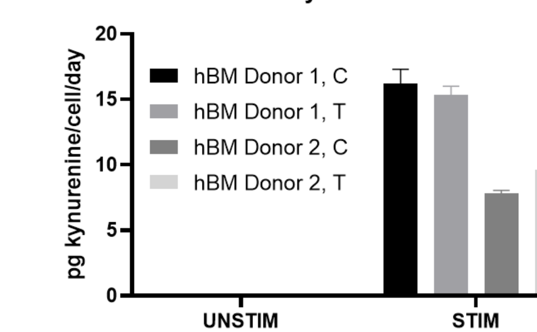
hUC-MSC Donor 1 ZsGreen expression at Final Product PDL

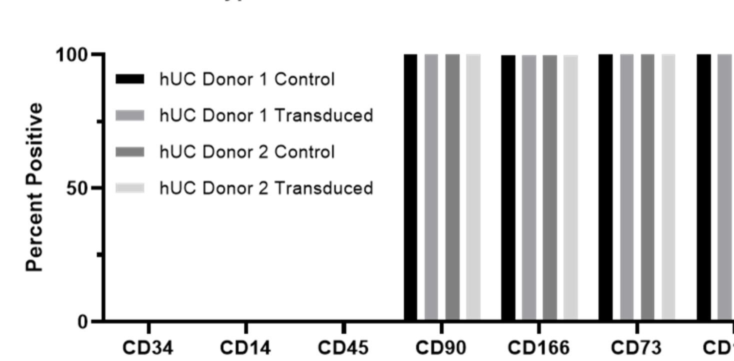


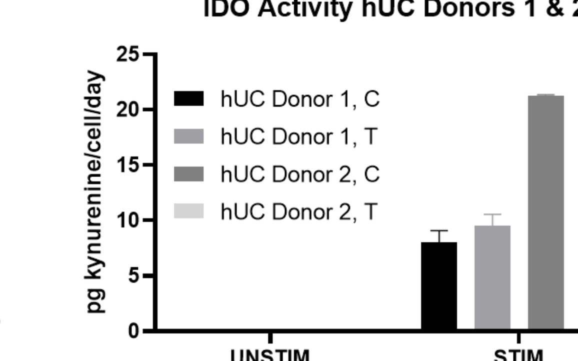



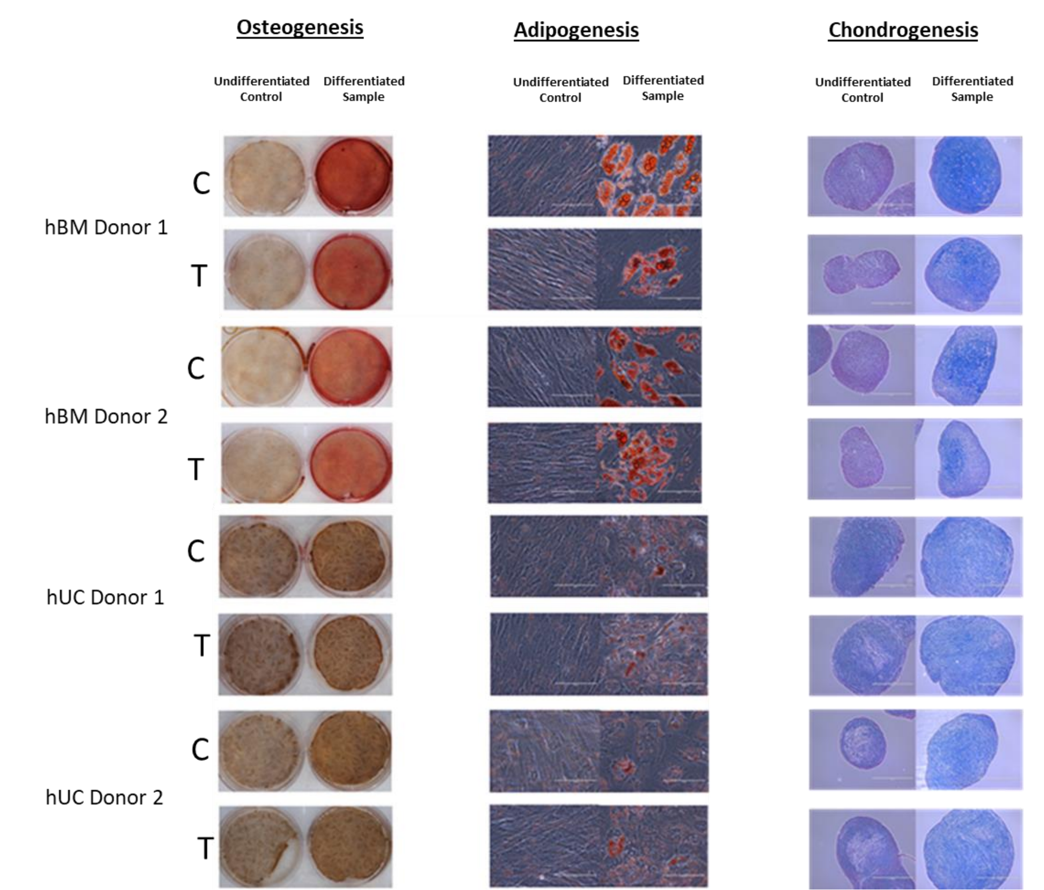

- MCBs of 2 hUC-MSC lots and 2 hBM-MSC lots were efficiently transduced with lentiviral vectors encoding ZsGreen using RoosterGEM™ (C=Control, T=Transduced).
- Harvested cells at Final Product PDL exhibited >95% ZsGreen positivity by flow cytometry, illustrating the robustness of RoosterGEM™ and the manufacturing process.
- At passage (MCB+1) transduced cells ( ) achieved fewer population doublings than control cells, but similar growth profiles were observed between control and transduced cells in subsequent passages.



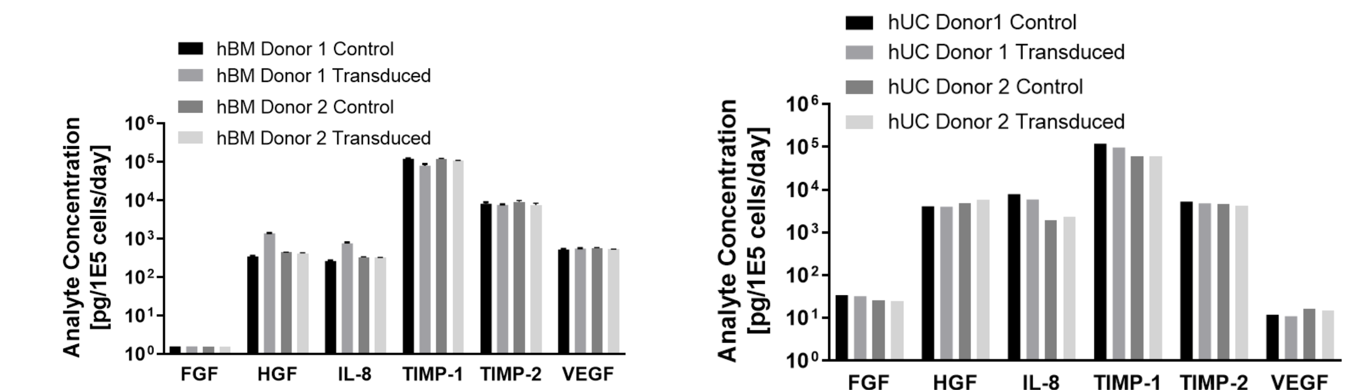




Phenotypic cell surface markers and trilineage differentiation of transduced (T) hMSCs compared to control (C) cells.




hMSC functional attributes were maintained in transduced cells (T) as shown by comparable inducible indoleamine 2, 3- dioxygenase (IDO) activity when stimulated with interferon gamma (INFγ) and angiogenic cytokine secretion profiles.

## CONCLUSIONS

- A novel chemically defined Genetic Engineering Medium has been developed for hMSCs that simplifies genetic modification within the context of cGMP hMSC manufacturing. This medium will be launched as RoosterGEM™.
- RoosterGEM™ has been used to transduce both hUC and hBM-MSCs with lentiviral vectors encoding ZsGreen or GFP, with initial transduction efficiencies 3-10X higher than leading expansion media.
- Critical process parameters (CPPs) required to optimize on a project basis are virus MOI and vendor, gene-specific promoter, as well as MSC tissue source and donor profile.
- A process to manufacture stably transduced hMSC working cell banks was developed with final product hMSCs having > 95% transgene expression, while maintaining typical MSC Critical Quality Attributes.
- This work establishes an optimized genetic modification unit operation within the context of a scalable xeno-free manufacturing process to reproducibly generate populations of genetically modified hMSCs.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