

Evolving the bio-safety of pluripotent stem cell based therapies

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The issues



Procurement

Efficacy

Fitness

Assurance

Procurement

- Donor consent/defining intent.
- Donor screening/testing (HIV, Hep B/C, others?)
- Tissue sourcing

Procurement of eggs/embryos?

Potential to conflict with donor

➤ IVF cycle

➤ Clinically failed IVF

- Immature @ ICSI/IVF
 - Failed to fertilise
 - Multi-pronucleate
- (10-30% Cycle)

➤ Fertile non-IVF

- Cadaveric
 - Caesarian
 - Elective Sterilisation
- (Ovarian follicular aspiration/growth)

➤ Induced from adult

➤ Interspecies

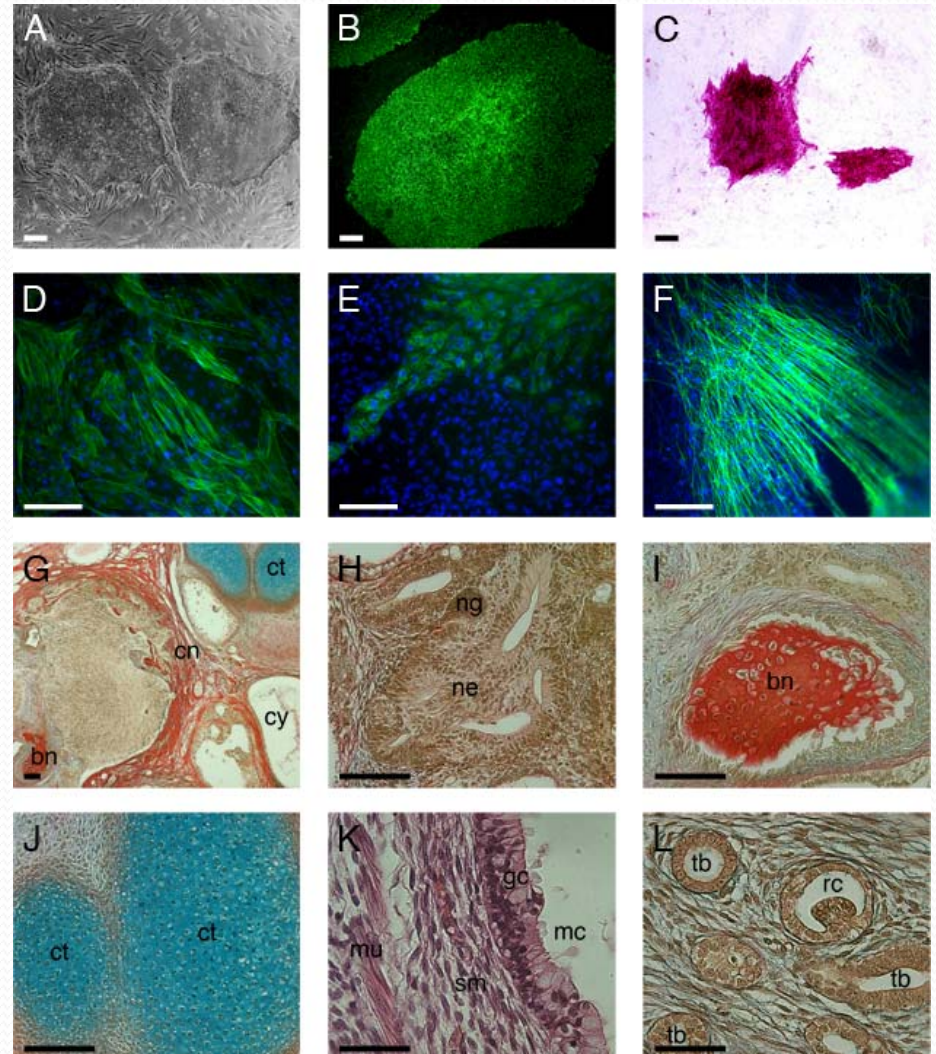
Artificial activation of failed eggs* ...

	No. eggs activated	No. Cleaved (%)	No. blastocysts	(%) blastocysts per cleaved
Failed IVF eggs	348	199 (57%)	14	(7)
Failed ICSI eggs	124	72 (58%)	4	(5)
In vitro matured eggs	47	20 (42%)	2	(10)

* Ca-ionophore/6-DMAP/CHX applied 24 h post insemination or 48 h post IVM.

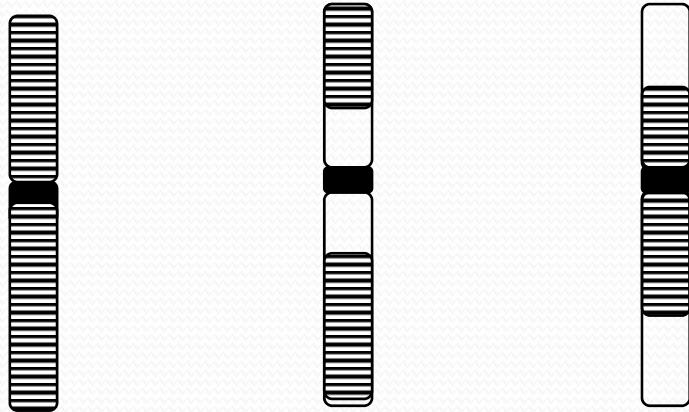
Sneddon, De Sousa, Kimber and Brison (submitted)

Capable* of “normal” embryo stem cell line (RCM1)



* Failed IVF/Parth (1/8)

A Parthenogenetic vs “Recovered” IVF embryo stem cell line?...



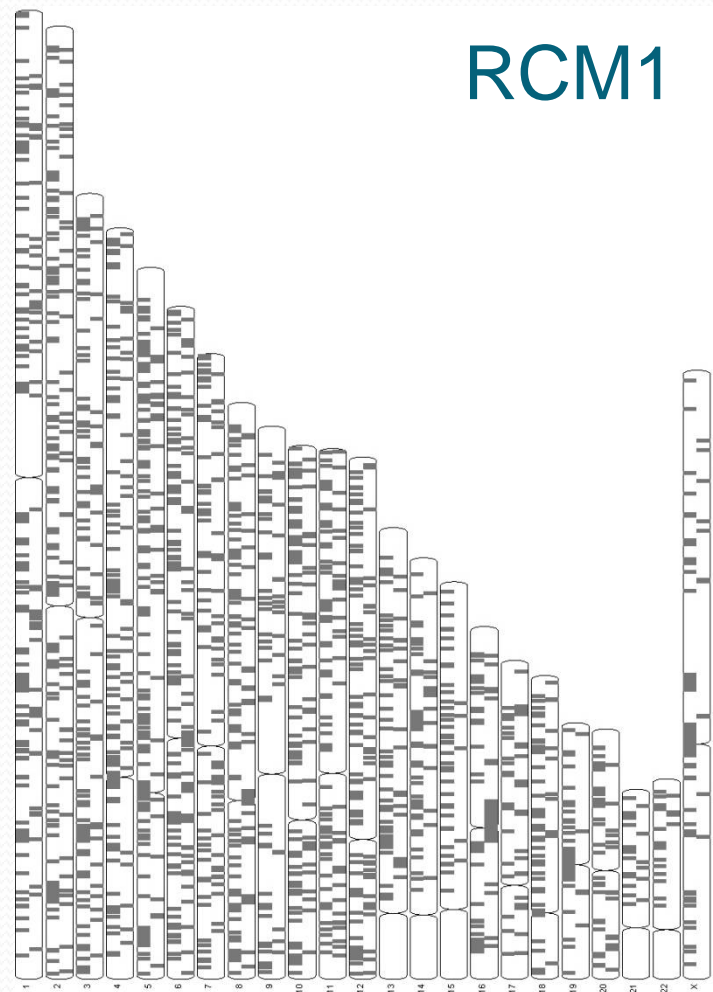
Fert.

Parth (MII)

Parth (MI)

Distribution of genetic heterozygosity assessed by genome-wide scan of Single Nucleotide Polymorphisms (SNPS)

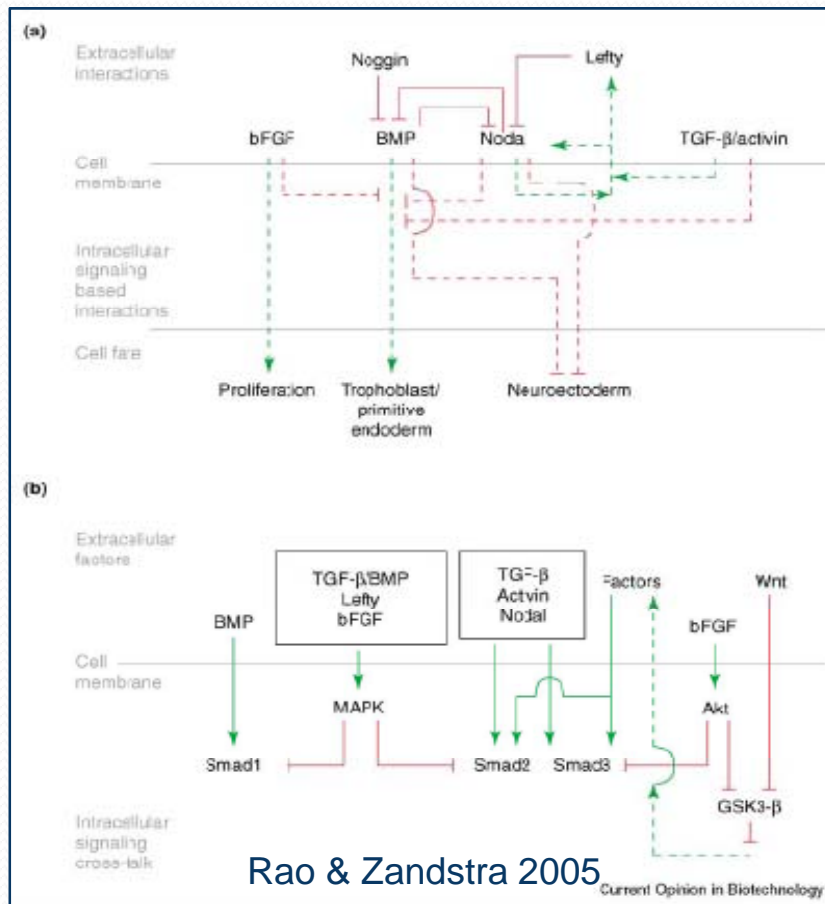
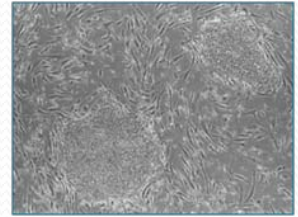
Kim et al., 2007 Cell Stem Cell



Efficacy

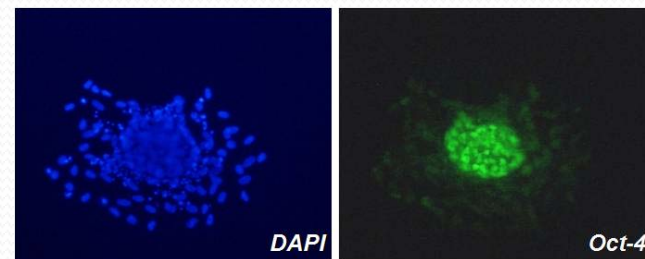
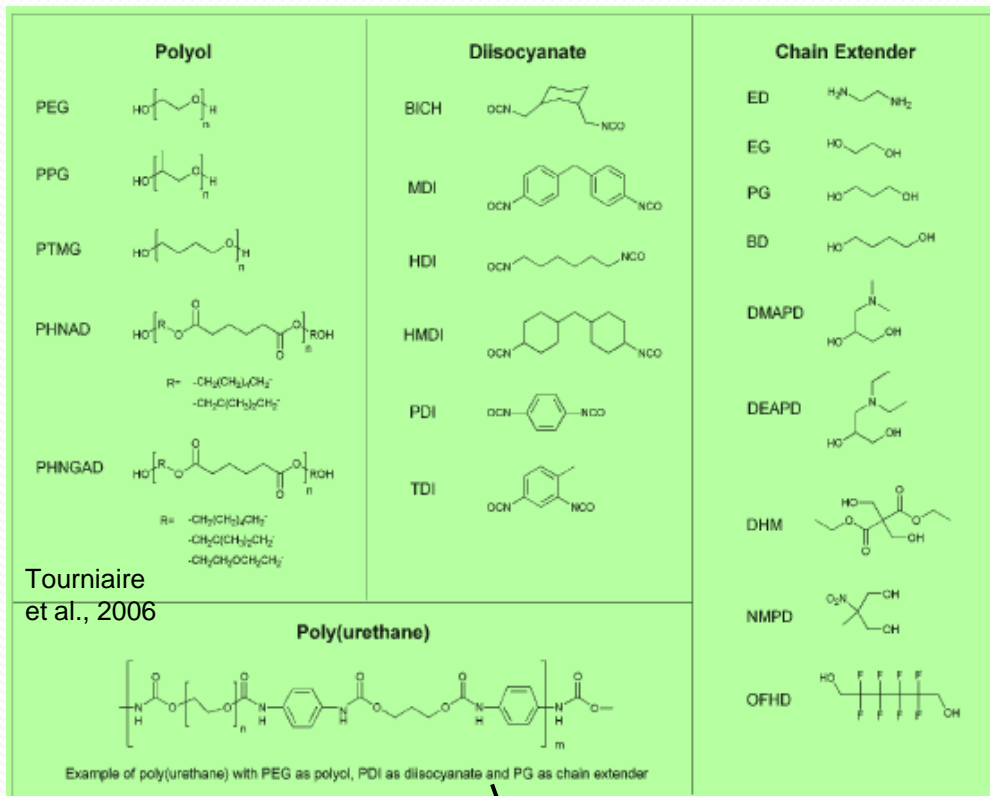
- Independence from animal/human cell products/chemical definition.
 - Biological Variance.
 - Zoonosis – cross-specific pathogenesis.
 - (ie. Athrax/Hantavirus/Q-fever)
 - Creutzfeldt-Jakob.
 - Endogenous retroviruses.
 - Immunogenic factors.
 - Non-human sialic acid

Regulation of human (embryo) Pluripotent stem cell maintenance.... (control of renewal vs diffn./survival & growth)



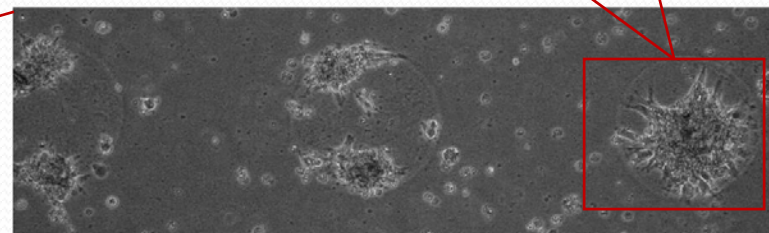
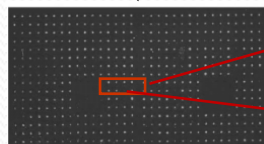
- Autocrine/paracrine signalling
- Signalling cross-talk
 - ✓ FGF
 - ✓ BMP/Noggin/Nodal/Lefty
 - ✓ TGF-β/activin
 - ✓ Wnt
 - ✓ IGF
 - ✓ S1P/PDGF (GPCRs)
- ECM (?)
 - ✓ Matrigel
 - ✓ Ln
 - ✓ Fn
 - ✓ Ln/Fn/Vn/Col IV

Screening to optimise definition and specificity of matrix supporting hESC attachment (7days).



Library
Printing

Array
Slide

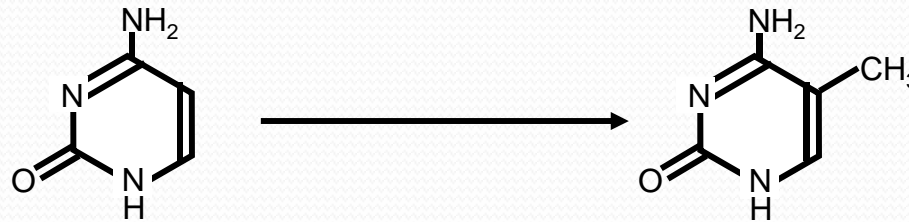


Fitness

- Functionality
- Stability
 - Genetic
 - Epigenetic
- Infectability

DNA Methylation and CpG Islands

- Transcriptional silencing of CpG clusters (~1000 bp) in promoters.
 - Cancer
(ie. p53 Tumour suppressor)
 - Paternally/maternal imprinting in development.
(ie. *H-19*, *SNRPN*, *MEG3* and *IGF-2*).



Cytosine

5-Methyl Cytosine

Genome-Wide Me-CGI Scanning in hESCs

Human ES Cell genomic DNA



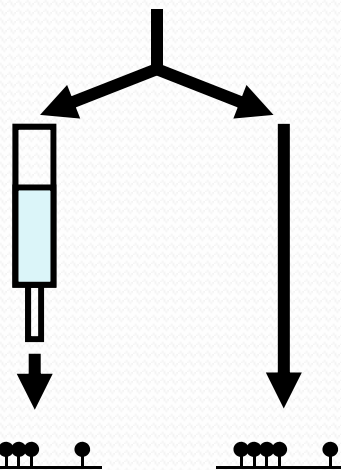
Mse I digested / Linker ligation



● = CpG

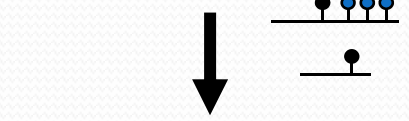
● = Methyl-CpG

*MeCP2-Sepharose Column
Affinity Purification*

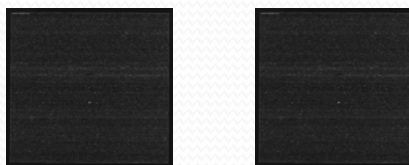


Total Input

*MBD-purified and total input DNA amplified
& labelled with Cy3/Cy5*



Hybridised to CGI array



Variation in hESC epigenetic profile

- No strict correlation between DNA methylation & silencing.
- Variation in X-inactivation.
 - “frozen developmental states?”
- Widely differing epigenetic states compatible with hESC phenotype.

Pathogen susceptibility -Prion diseases?

- Degenerative CNS disorders caused by transmissible pathogenic isoforms of prion protein (PRP).
 - Animal (Scrapie/BSE/CWD) & Human (CJD/Kuru) forms.
- Creutzfeldt-Jakob Disease (CJD) most common in humans (0.5-1.5 cases/million per year).
- CJD forms: (Different etiology /epidemiology / manifestation)
 - Sporadic (85%) – random misfolding? Spontaneous mutation?
 - Familial (10-15%) – mutations.
 - Iatrogenic (1%) – inadvertent human to human.
 - Variant (UK & France) – bovine to human.

HESC susceptibility to prion diseases?

Requires:

- ✓ Genetic susceptibility polymorphisms.
- ✓ Expression of constitutive normal PRPc isoform.
- ✓ Tissue/cell-specific susceptibility to infection.
(uptake, amplification, transmission)
- ✓ Exposure to pathogenic isoforms (variant/iatrogenic).
ie. bovine/human sourced reagents in culture.



Assurance

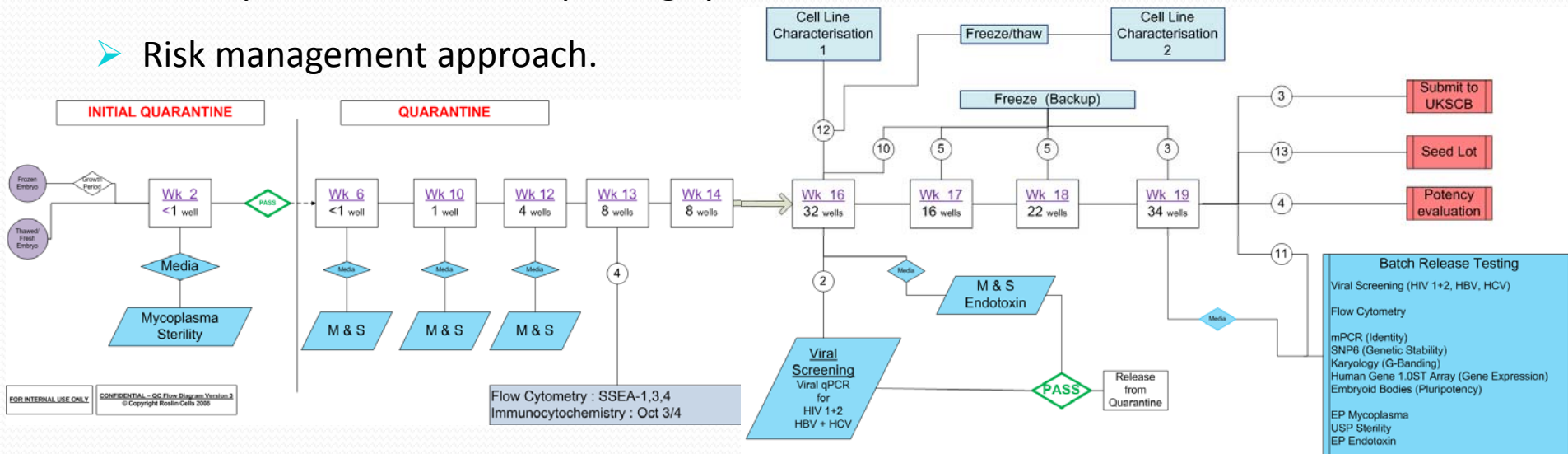
**Quality Assured
Good Manufacturing Practice**

Progress

- 6 “Clinical grade” hESC – ESI Singapore (2007)
 - Reliance on research grade reagents.
 - Usage of animal sourced products (Traceable).
 - Non-cGMP subclones available for R&D.
- U.S. FDA approval of qualified research grade hESCs for therapy.

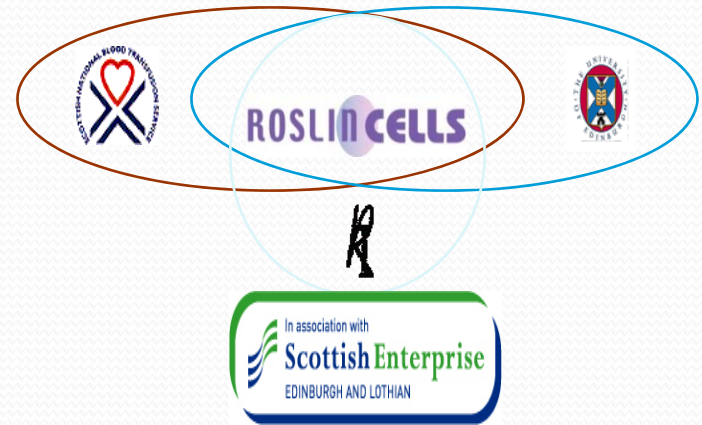
Quality Assured Good Manufacturing Practice

- Compliance with requisite regulatory standards to ensure safety
(based on evolving pharmaceutical/blood & tissue transplantation service standards)
- Approved documentation & records system providing full traceability.
- Staff training system.
- Internal/external audit & supplier approval.
- Qualification & validation of equipment, facilities, processes, Quality Control assays.
- Quality related incident reporting system.
- Risk management approach.



Roslin Cells

- Operating since 2006
- 14 full time staff
- Located at Roslin BioCentre
- Dedicated Grade A/B clean room/ R&D/QC labs
- Licensed for the derivation of hESCs by the HFEA
- Licensed for the processing, storage etc of tissues for human application by the HTA
- ISO9001 accredited.
- Confirmed by independent audit to be operating to Quality Assured Good Manufacturing Practice.



Summary....

Procurement
Defusing Conflict

Efficacy
Chemical
Definition

Fitness
Function
Stability
Infectability

Assurance
GMP
Traceability

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