protocol adapted for monkeys) allowed the human oocyte to develop to the 8 cell stage (blastocyte). But they had very small ICMs therefore did not support ESC derivation, hence the need for more optimisation. The addition of caffeine drastically improved development, showing more prominent ICMs and 50% gave rise to ESC- like colonies forming NT-ESC lines. These lines showed expression of pluripotency markers, sox2, Oct4 and Nanog. Nuclei of fibroblasts were taken from a 35 and 75 year old. Both in cell culture could form embryoid bodies, and when injected into SCID mice, formed tumours containing cell types from all three germ layers!

18. Can form human nuclear transfer ESCs from patient specific somatic cells. This would have no problems of rejection and could be used to study disease mechanisms and develop specific therapies.
19. H3 K9me3, 5mc
20. H3 K4me3, K9ac
21. Trying to iPSCs from differentiated cells (no need for oocyte) via viral vector transfection of SC-associated genes. Originally 24 genes were identified as important in ESCs.. by removing one factor from transfection each time found which were important. Oct4, sox2, klf 4, cMyc.
22. Very inefficient in human cells… only 0.01-0.1% success rate. The transformation was also not 100% complete as retained some features of previous cell.
23. Viral vectors.. can reactivate cancer genes, and DNA inserted is there forever. Use plasmids as non- insertional avoiding insertional mutagenesis… not as effective though. Even better, delivery of factors as whole cell extracts or purified proteins or modified small RNA molecules. (need improvement of this transgene-free approach)
24. Patient with disease… take skin biopsy and iPSCs for cell therapy can take these cells and repair the disease causing mutation by gene targeting. These are now fixed iPSCs which can differentiate into cells from all three germ layers. Differentiate into patient specific replacement of damaged cells and transplant in healthy ones. For disease modelling can keep the unfixed iPSCs and differentiate into the disease affected cell types and screen to see if any drugs help which can be used to treat the patient.
25. Differentiation strategies are at present deficient… it's hard to get the correct cell type in a pure cell culture. Diseases often have more than one factor involved/ can be late-onset. Is it possible to model this in vitro?
26. Jaenisch in 2007. Cured an anaemic mouse of sickle cell anaemia using iPSCs from the mouse's fibroblasts and correcting the sickle cell mutation.
27. Ads: Readily available
    Patient specific so no transplant rejection
    Used to model genetic disease
    Few ethical issues
    Unlimited proliferative ability
    Pluripotent so can form most cell types.
    Dis: low reprogramming rate and incomplete reprogramming
    Can form tumours
    Genetic mutations during long term culture