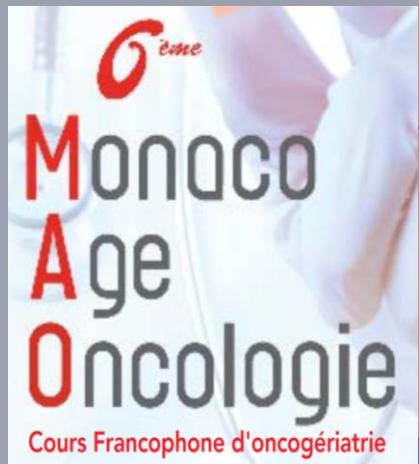
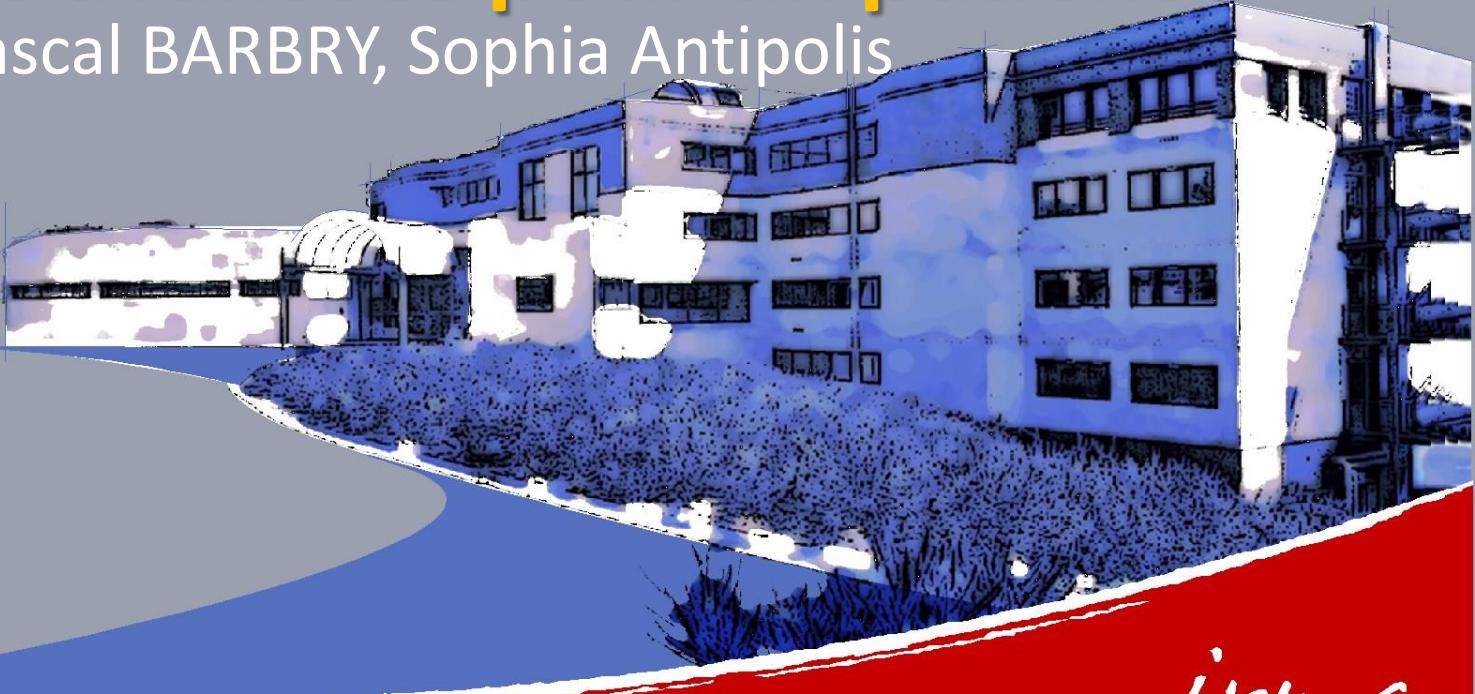


INSTITUT DE PHARMACOLOGIE MOLÉCULAIRE ET CELLULAIRE

De la technique aux découvertes : quelles avancées pour un patient ?

Pascal BARBRY, Sophia Antipolis



ipmc

INSTITUT DE PHARMACOLOGIE MOLECULAIRE ET CELLULAIRE



20 groupes de recherche de niveau international, >20 M€ de budget annuel

210 chercheurs, ingénieurs techniciens, étudiants de 20 nationalités différentes
(+ 30% au cours des six dernières années)

De 2011 à 2016 : plus de 500 publications dans les plus grandes revues scientifiques (*Nature*, *Science*, *Cell*, *New England Journal of Medicine*), 27 brevets publiés, 341 invitations à conférences, 66 organisations de congrès, 1 Startup hébergée(E-Phy-Science)

3 labex, 1 infrastructure nationale biotechnologie/santé, 3 FHU : OncoAge, InovPain, IRIHE

3 plateformes IBISA, dont 2 certifiées ISO9001

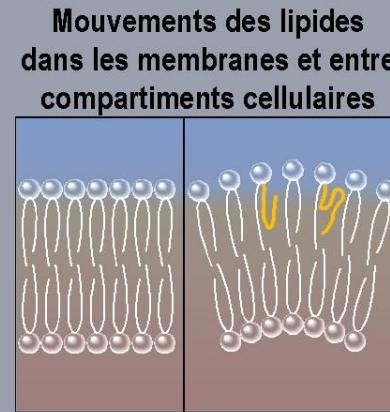
1 Médaille d'Or et 3 Médailles d'Argent CNRS, 1 Académicien des Sciences, 1 correspondant étranger

Collaborations industrielles (Moleac, GSK, ONO, Innate Pharma, Galderma, Immunosearch, TXCell, Boehringer) SATT-N, SATT-SE, Canceropôle PACA

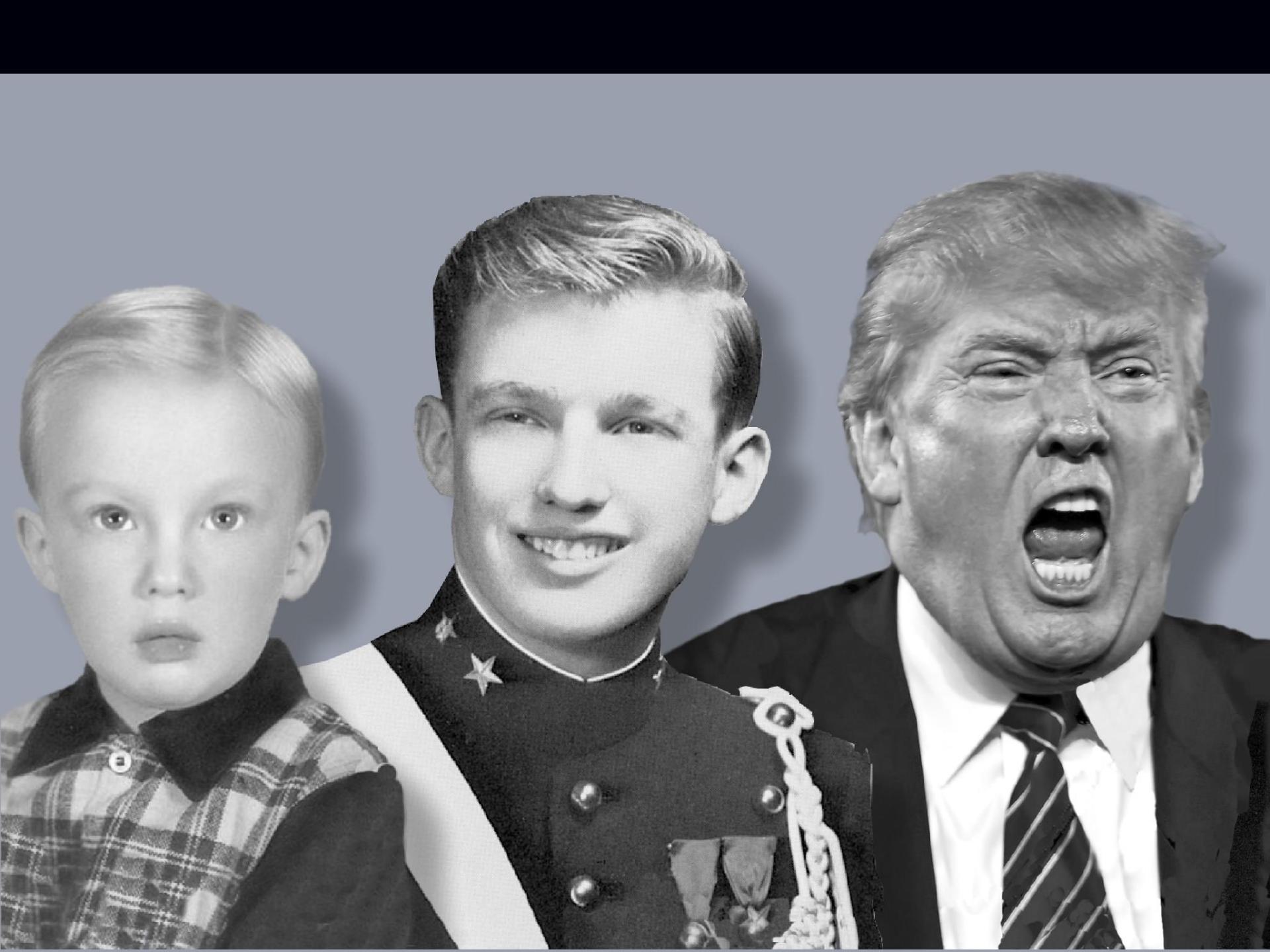


ipsmc

- **Canaux ioniques** (douleur, perception, mécanosensibilité, accident vasculaire, épilepsie)
- **Signalisation moléculaire et cellulaire** (trafic membranaire, lipides, neuroinflammation, fibrose pulmonaire, mucoviscidose, cancer)
- **Maladies neurodégénératives**
- **Génomique fonctionnelle, bioinformatique**



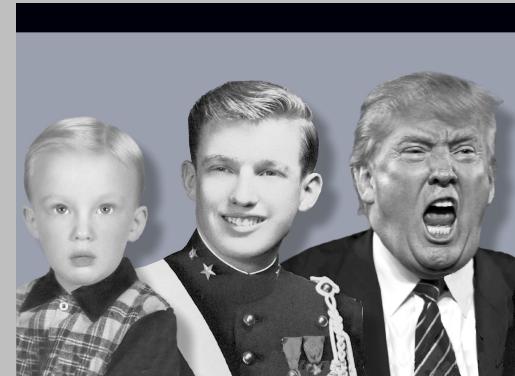




Modern biological theories of aging in humans

Two main categories:

- **Programmed theories.** Aging follows a biological timetable, perhaps a continuation of the one that regulates childhood growth and development. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair and defense responses.
- **Damage or error theories.** The damage or error theories emphasize environmental assaults to living organisms that induce cumulative damage at various levels as the cause of aging.



The programmed theory

Three sub-categories:

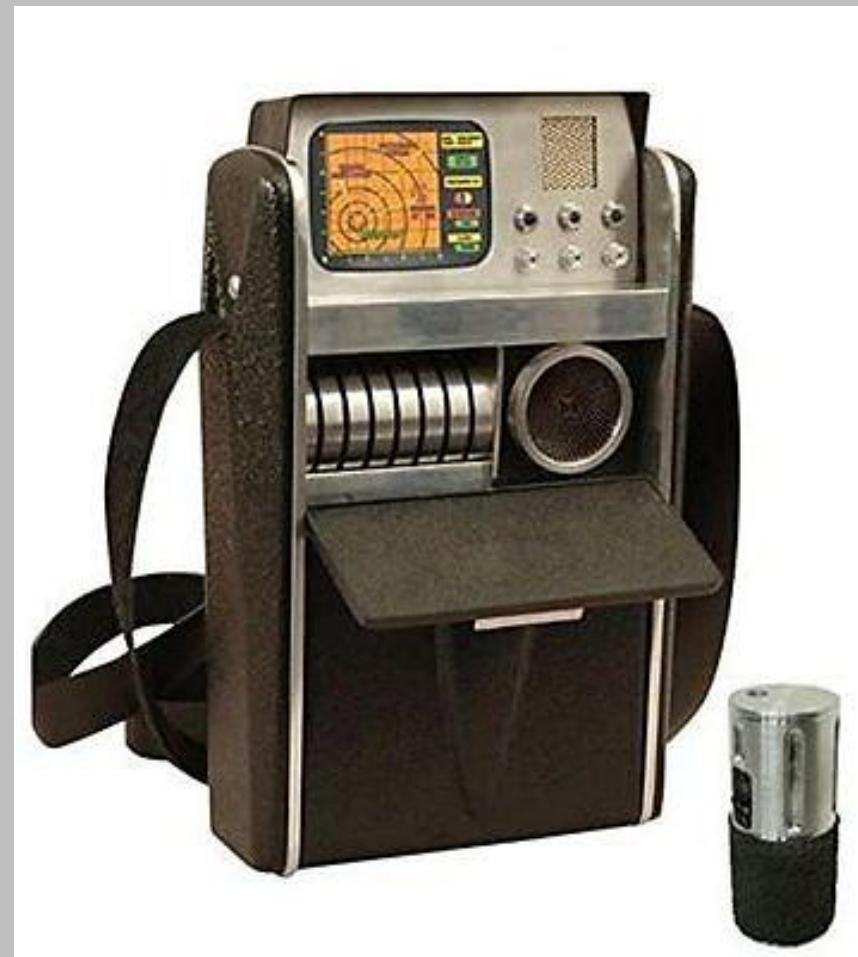
1. **Programmed Longevity.** Sequential switching on and off of certain genes, senescence being defined as the time when age-associated deficits are manifested.
2. **Endocrine Theory.** Biological clocks act through hormones to control the pace of aging. Evolutionarily conserved role of insulin/IGF-1 signaling (IIS) pathway.
3. **Immunological Theory.** The immune system declines over time, which leads to an increased vulnerability to infectious disease and thus aging and death. Dysregulated immune response has been linked to cardiovascular disease, inflammation, Alzheimer's disease (AD), and cancer.

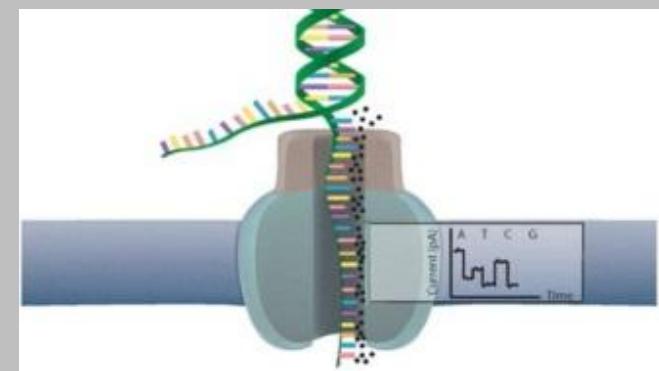
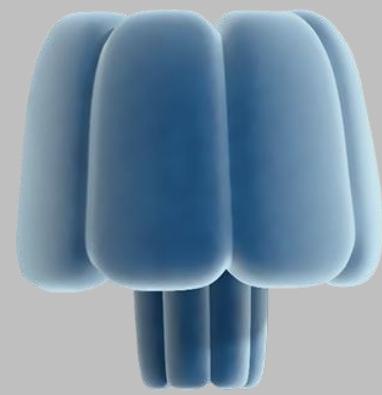
The damage or error theories

1. **Wear and tear theory (Weismann, 1882).** Cells and tissues have vital parts that wear out with time.
2. **Rate of living theory (Rubner, 1908).** Inverted relationship between organism's metabolism and life span.
3. **Cross-linking theory (Bjorksten, 1942).** An accumulation of cross-linked proteins damages cells and tissues.
4. **Free radical theory or oxidative damage theory of ageing (Gerschman & Harman, 1954-1955).** Accumulation over time of damages to biomolecules by superoxide and other free radicals.
 - Single- and double-strand breaks in nucleic acids; chemical crosslinks.
 - Importance of several signaling pathways: ROS signaling; antagonisms between growth (TOR) and stress resistance (fork head box O transcription factors); AMP-activated protein kinase; sirtuins...
5. **Somatic DNA damage theory.** Continuous nuclear and/or mitochondrial DNA damages in living cells. Accumulation of genetic mutations with increasing age => deterioration + malfunction.



A flavor of Star Trek Tricorder



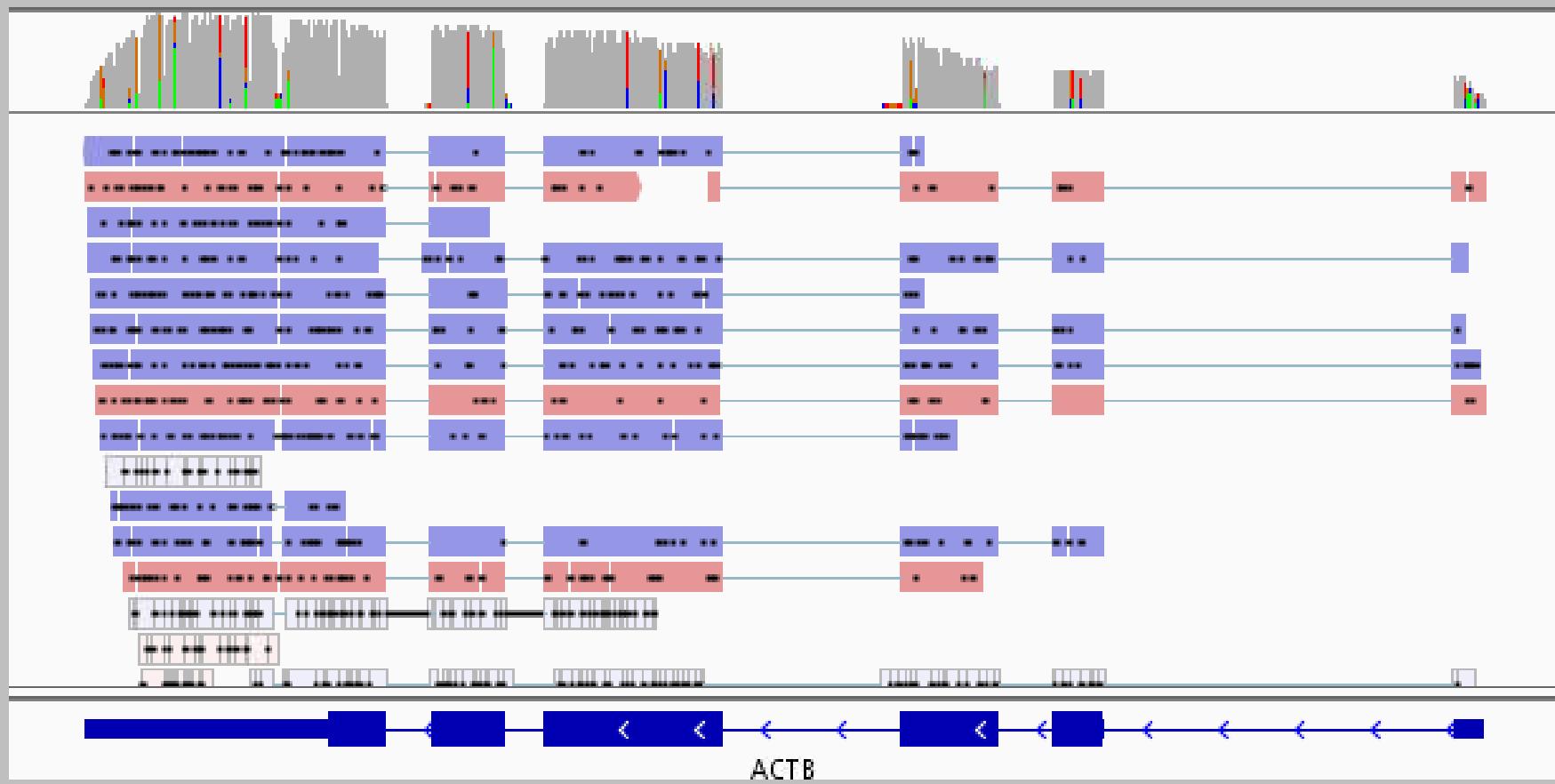


Portable (space, zika)

Long read sequencing (30kbp++)

>95% precision

Many applications: detecting microbes, haplotyping, methylation



A future « Minion » revolution in medicine

- A new tool for semiology:
 - Addressing more rapidly infectious diseases
 - Metagenomics
 - Long read information => haplotyping (combined or not with short read NGS)
 - Nucleic acid modifications → epigenetics, DNA modification
- Opening the systems biology box in any medical office
- But improvements still required (high error rate, sensitivity, bioinformatics,...) → short reads needed



Genetics of human longevity

- 25% of the variation in human lifespan thought to be caused by genetic variation (Herskind et al. 1996), especially after age 85 years (Hjelmborg et al. 2006).
- Candidate genes for longevity encode proteins engaged in different biological processes including lipoprotein metabolism and inflammatory processes (Christensen et al. 2006).
- Northern Europe:
 - Genetic variation in APOE, CETP, and IL6, and possible HSPA14 associated with human longevity (Soerensen et al. Evidence from case-control and longitudinal studies supports associations of genetic variation in APOE, CETP, and IL6 with human longevity. *Age (Dordr)*. 2013 Apr;35(2):487-500)
- China:
 - 11 independent loci, replicated in 2 studies (Northern + Southern China). Association with « immune response and inflammation », « MAPK », « calcium signaling » (Zeng et al, 2016. *Scientific Reports* | 6:21243 | DOI: 10.1038/srep21243)
- Alzheimer's disease:
 - Lambert et al. *Meta-analysis of 74, 046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet.* 45, 1452–1458 (2013) → APOE also!
 - Karch et al. *Alzheimer's disease genetics: from the bench to the clinic. Neuron.* 83, 11–26 (2014).

GWAS study:
Northern China: (1063 + 1115) centenarians
Southern China: (887 + 1412) controls

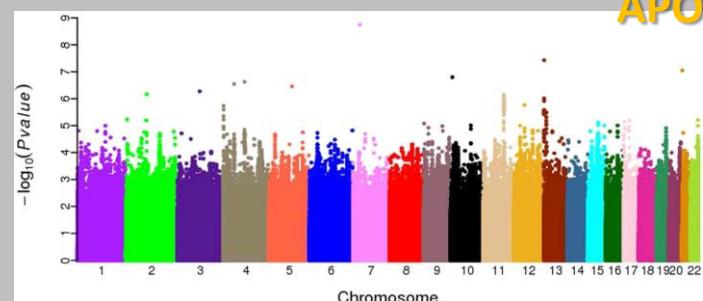
11 independent loci,
replicated in 2 studies

SNP	Chr.	Position	Nearest gene	Coded/ non-coded allele	Southern region of China			Northern region of China			Southern-Northern Combined			Meta analysis
					MAF (case/control)	P	Odds ratio	MAF (case/control)	P	Odds ratio	MAF (case/control)	P	Odds ratio	
rs2069837	7	22768027	<i>IL6</i> (intronic)	G/A	0.018/0.033	5.98E-03	0.582	0.086/0.134	1.00E-06	0.64	0.053/0.095	1.80E-09	0.61	4.05E-08
rs2440012	13	19440123	<i>ANKRD20A9P</i> (nc_exonic)	G/C	0.050/0.092	1.38E-06	0.506	0.057/0.079	2.26E-03	0.69	0.054/0.084	3.73E-08	0.602	4.89E-08
rs145672791	21	14750023	<i>MIR3156-3</i> (28kb downstream)	A/G	0.003/0.011	5.08E-03	0.267	0.004/0.022	9.88E-06	0.203	0.004/0.018	8.95E-08	0.219	2.34E-07
rs61856137	10	5087978	<i>AKR1C2</i> (27kb upstream)	T/G	0.019/0.032	9.85E-03	0.572	0.040/0.070	1.56E-05	0.549	0.029/0.056	1.60E-07	0.544	7.54E-07
rs2704588	4	89849772	<i>FAM13A</i> (intronic)	C/T	0.004/0.013	4.32E-03	0.289	0.005/0.021	3.26E-05	0.237	0.004/0.018	2.38E-07	0.248	5.63E-07
rs1487614	4	42269480	<i>BEND4</i> (114kb upstream)	T/C	0.112/0.146	1.85E-03	0.738	0.103/0.141	8.13E-05	0.707	0.107/0.143	2.87E-07	0.716	5.30E-07
rs10934524	3	96150160	<i>EPHA6</i> (383kb upstream)	T/C	0.453/0.384	2.97E-05	1.354	0.470/0.431	4.76E-03	1.192	0.462/0.413	5.33E-07	1.266	1.16E-06
rs57681851	4	2290698	<i>ZFYVE28</i> (intronic)	G/T	0.187/0.136	7.05E-05	1.448	0.155/0.128	7.75E-03	1.256	0.170/0.131	1.83E-06	1.348	3.78E-06
rs7213812	17	31448649	<i>ASIC2</i> (intronic)	C/A	0.216/0.161	1.36E-05	1.45	0.176/0.152	2.84E-02	1.182	0.196/0.155	6.33E-06	1.29	6.25E-06
rs9568833	13	53827016	<i>OLFM4</i> (200kb downstream)	T/C	0.145/0.193	7.85E-05	0.712	0.144/0.168	2.46E-02	0.836	0.144/0.177	1.77E-05	0.778	1.75E-05
rs405509	19	45408836	<i>APOE</i> (200 bp upstream)	G/T	0.374/0.316	7.92E-05	1.32	0.308/0.279	2.56E-02	1.148	0.341/0.293	3.64E-05	1.21	1.85E-05

Four pathways highly associated with longevity ($P \leq 0.006$) in Han Chinese:

- starch, sucrose and xenobiotic metabolism;
- immune response and inflammation*
- MAPK*
- calcium signaling*

* confirmed in other cohorts



Importance croissante de la génétique pour prédire la réponse à une thérapie ciblée en cancérologie

<i>Pathologie</i>	<i>Biomarqueur</i>	<i># tests</i>
Cancer du sein	Amplification d'HER2	8924
Cancer de l'estomac	Amplification d'HER2	709
Cancer colorectal	Mutations de KRAS	19 347
	Mutations de NRAS	3330
GIST	Mutations de KIT	1105
	Mutations de PDGFRA	1005
Cancer du poumon	Mutations d'EGFR	23336
	Translocation d'ALK	18861
Mélanome	Mutation de BRAF V600	5026
Leucémies	Détection de BCR-ABL	6750
	Mutations d'ABL	861
		TOTAL: 89254

Marqueur	Nombre patients	% altérations moléculaires	Non interprétables
Mutations EGFR **	23336	10.0%	8.0%
Translocation ALK *	18861	3.5%	13.4%
Mutations KRAS	22958	27.0%	7.9%
Mutations BRAF	20100	2.0%	8.9%
Mutations HER2	17843	0.7%	10.1%
Mutations PI3KCA	17375	2.4%	10.4%

** *gefitinib, erlotinib, afatinib*

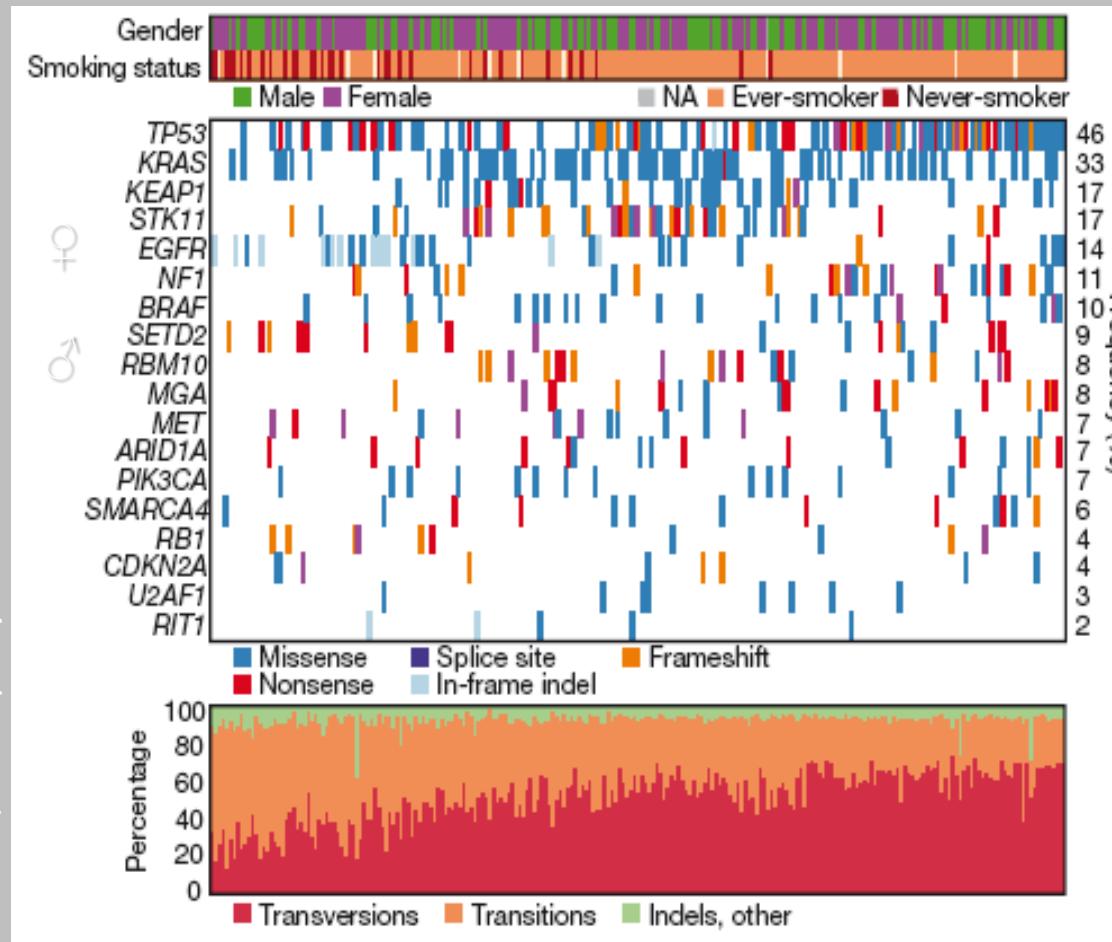
* *crizotinib, ceritinib*

Données Plateformes génétiques INCa, 2013

Comprehensive molecular profiling of 230 lung adenocarcinoma

ARNm, microARN, mutations, variations du nombre de copies, méthylation, protéomique

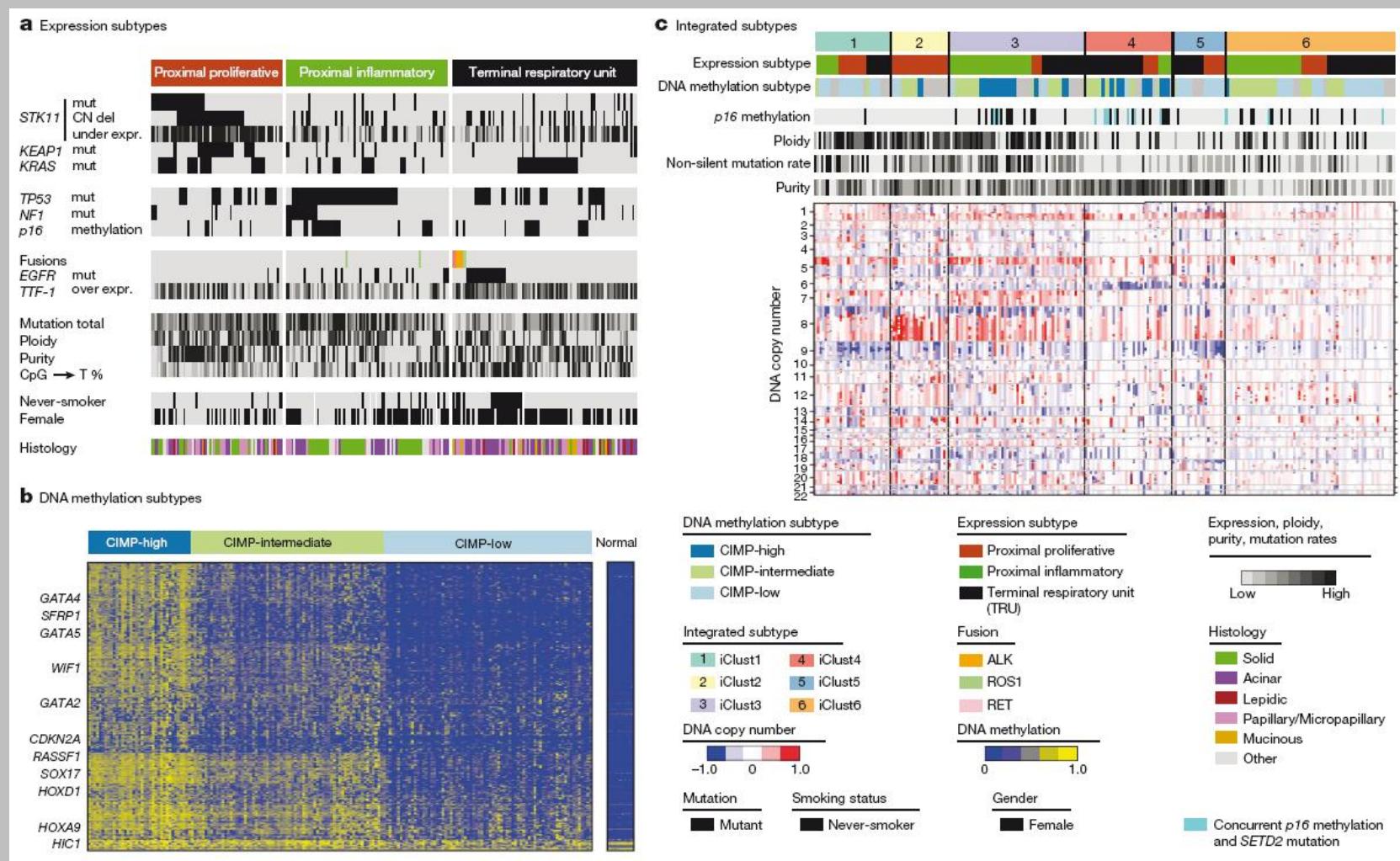
EA Network + Cancer Genome Atlas Research
Nature (2014)



Taux élevé de mutations somatiques: 8.9 mutations par mégabase

“Comprehensive molecular profiling of 230 lung adenocarcinoma”

ARNm, microARN, mutations, variations nombres copies, méthylation, protéomique

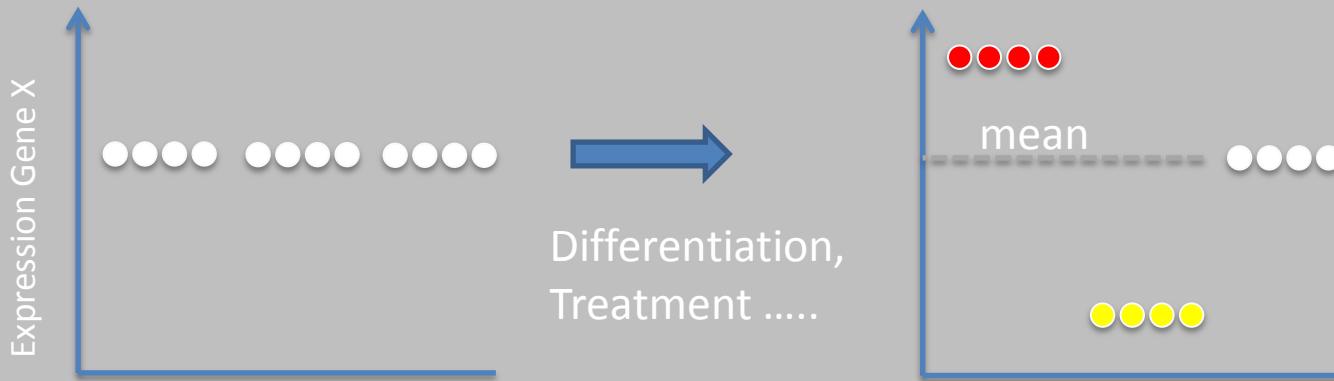


EA Collisson + Cancer Genome Atlas Research Network, Nature (2014)

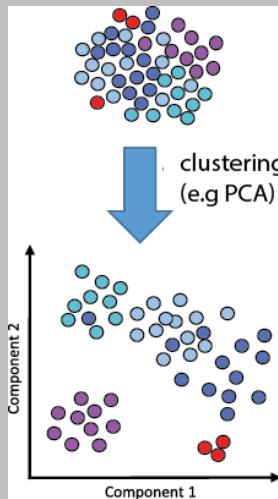
Cellular theories of aging

- Cell division gradually slows at each successive division, until replicative **senescence**, at which point no further divisions will occur.
- Increasing proportion of senescent cells over time (terminal stage at which cells cease to divide).
- The mechanism of replicative senescence is thought to involve some type of biological clock within the cell, which measures the number of cellular divisions and signals the cell to discontinue division at some genetically predetermined time → Hayflick limit theory of aging (number of division until stop linked to the shortening of telomeres at each cell division until a critical length)

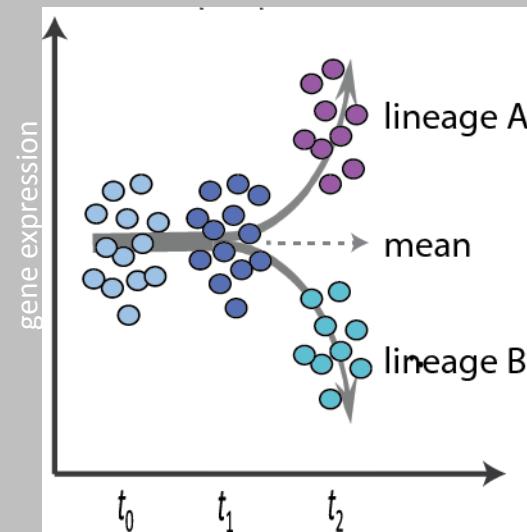
Single cell RNA analyses



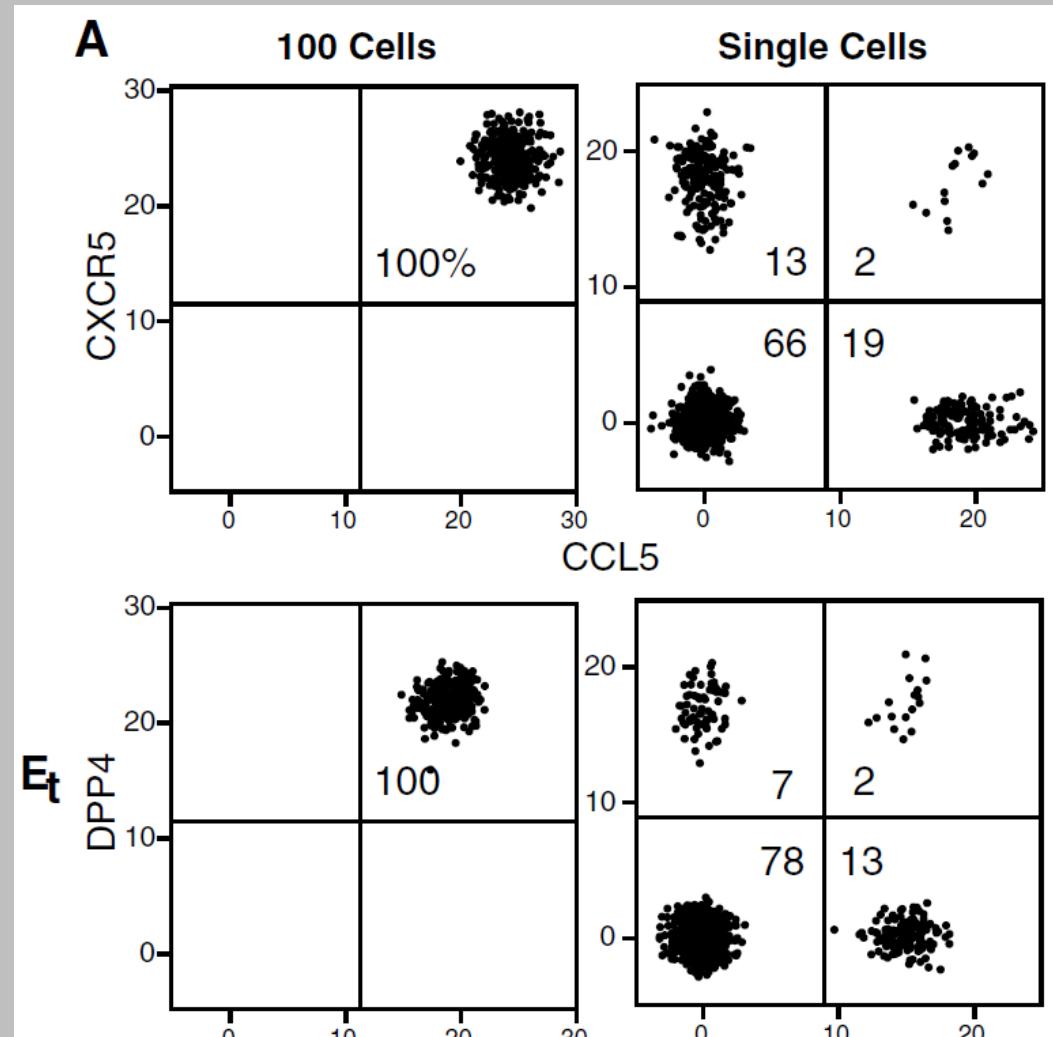
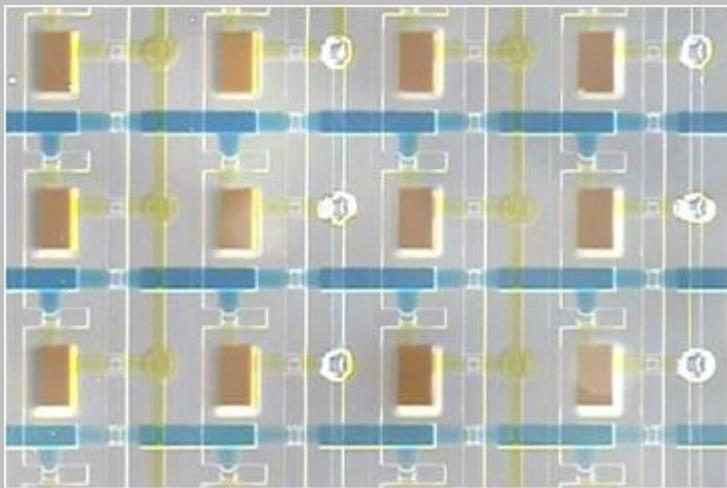
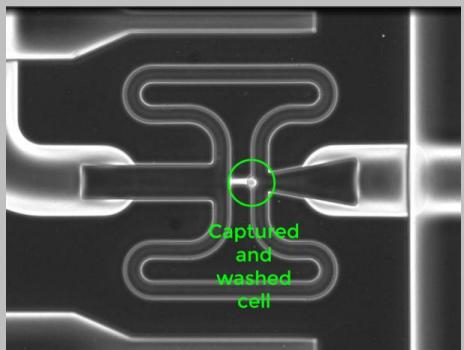
- Population sequencing yields average values
- Changes in subpopulation



- Identification of cell types or cell states

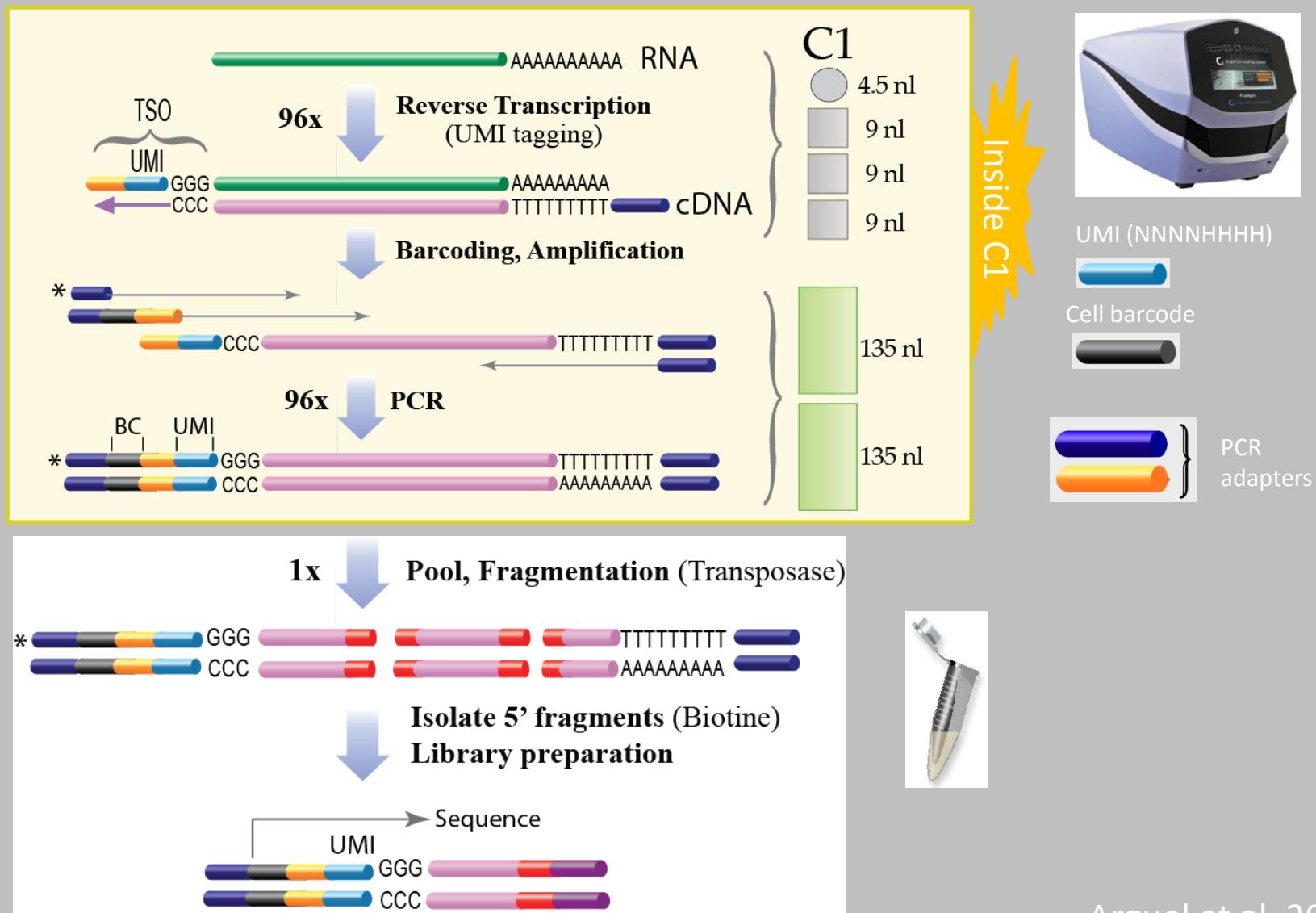


The challenge of the measurement at a single cell level

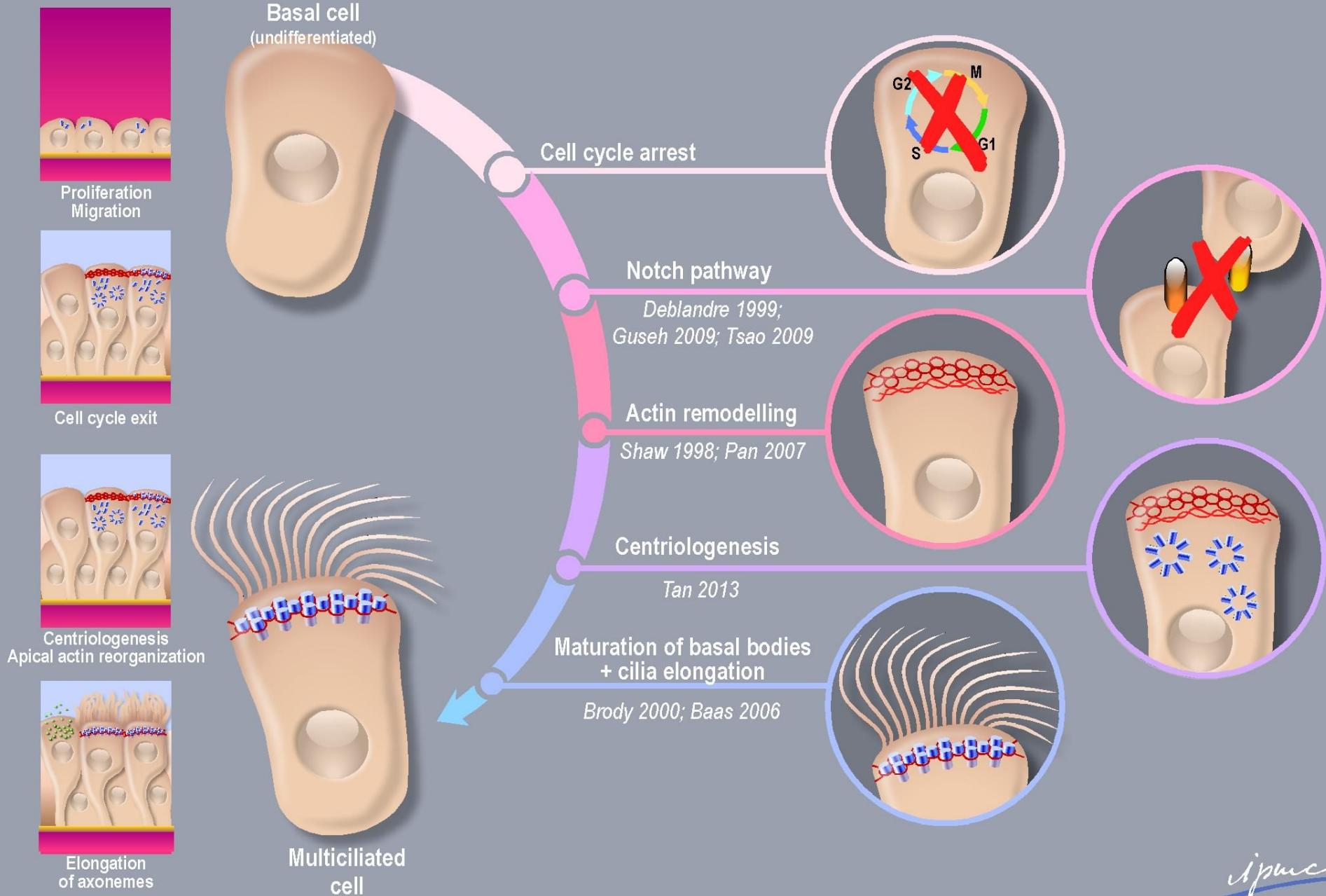


Dominguez et coll (2013) Journal of Immunological Methods

Modified smart-seq protocol: *On-Chip Barcoding + UMI*

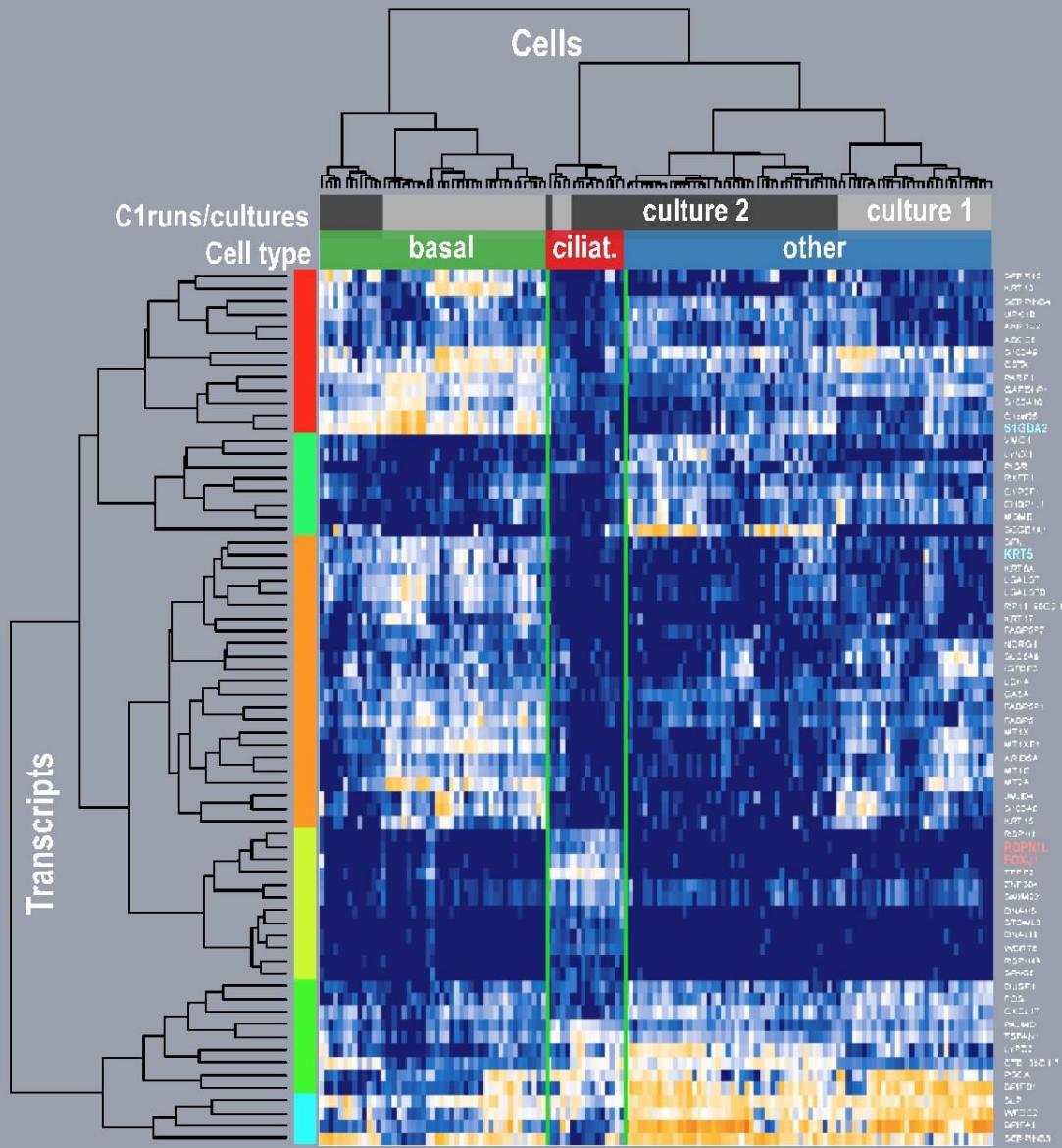
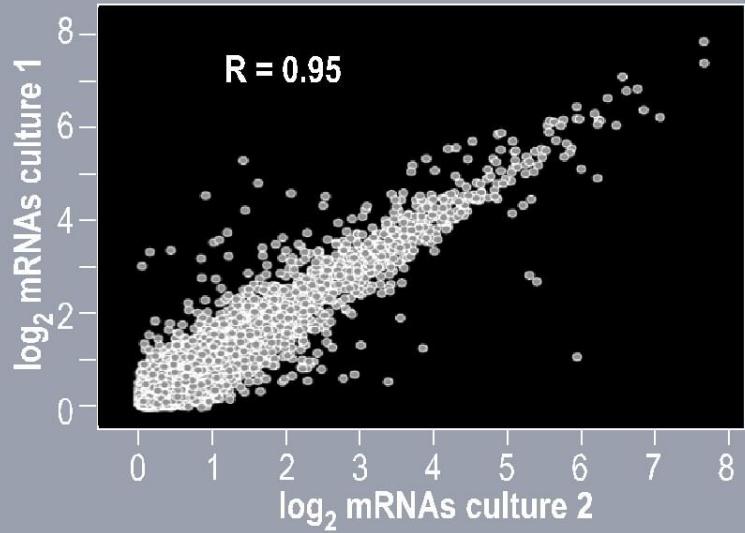
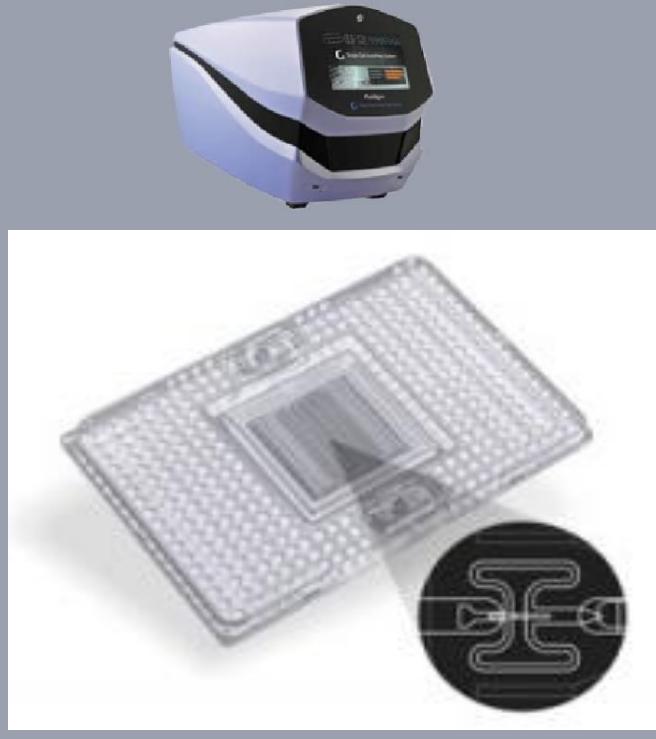


The different steps of MCC differentiation



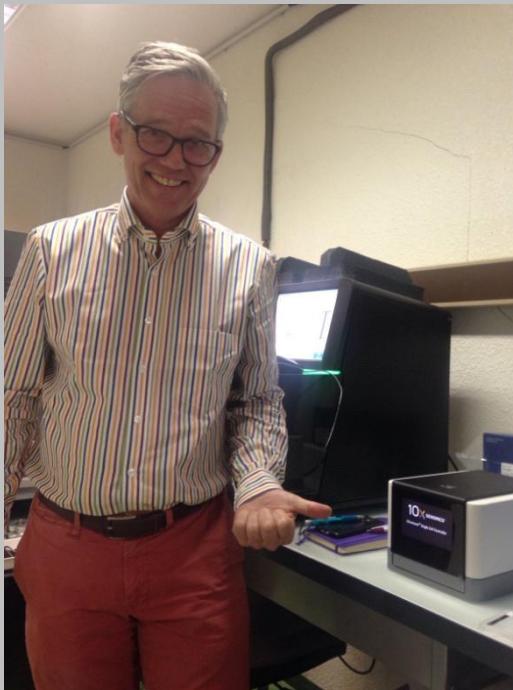
ipmc

An identity card for each human nasal epithelial cell

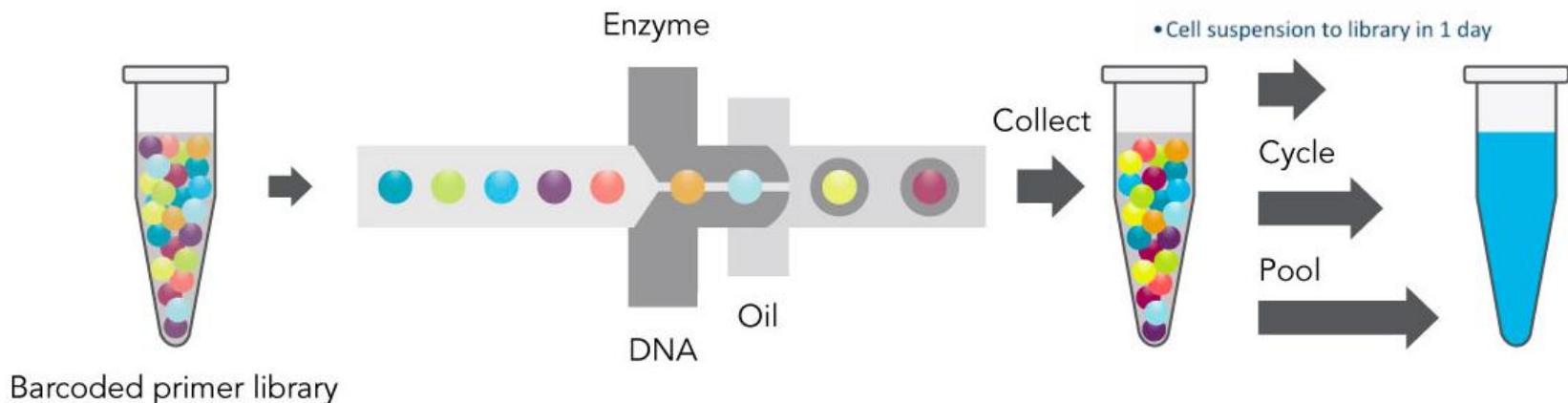


Arguel et al, 2016, NAR

ijmuc

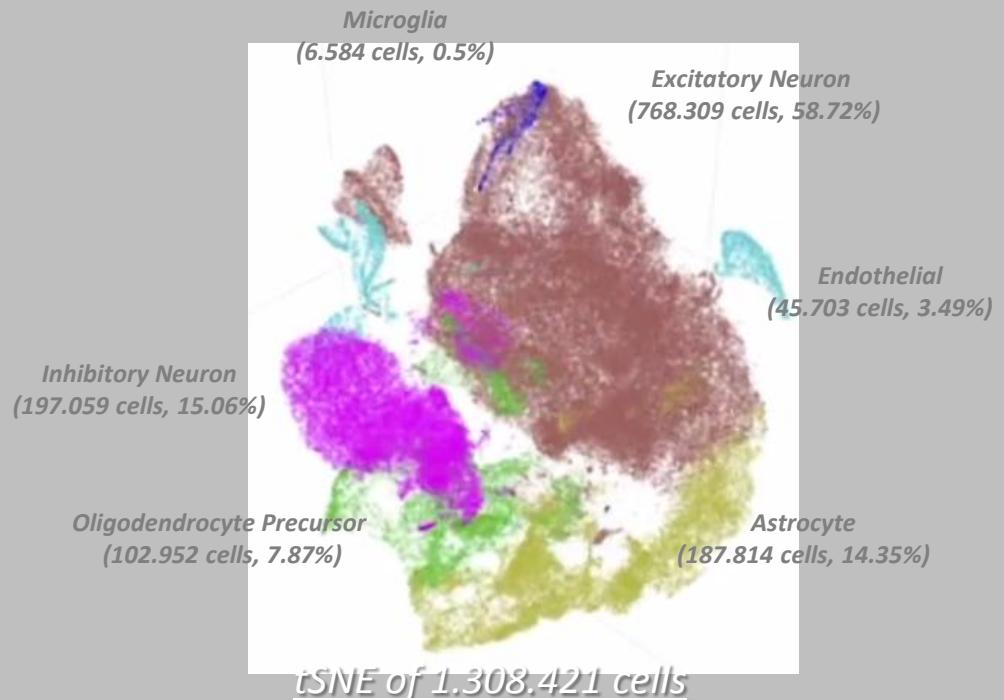
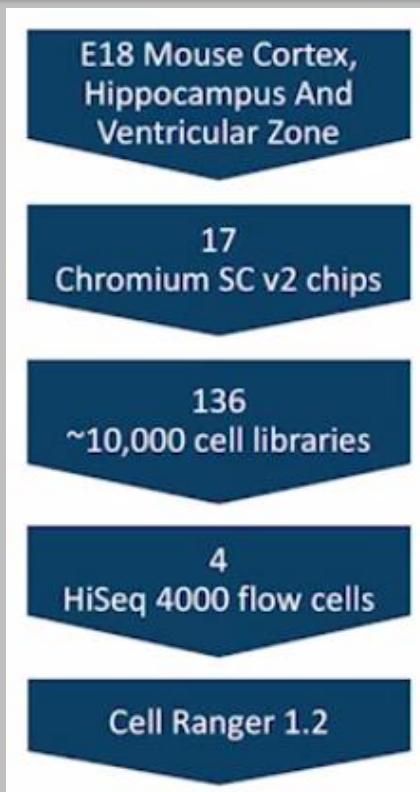


- Standard sequencing configurations
- Easier to multiplex with non-SC libraries
- High quality UMI and Cell Barcode reads
- High performance on patterned flowcells
- Partitions 100 - 10,000+ cells per channel in < 7 minutes
- Recovers ~65% of all loaded cells
- Low doublet rate: 0.9% per 1,000 cells
- Optimized reverse transcription and cDNA clean-up
- Enzymatic fragmentation

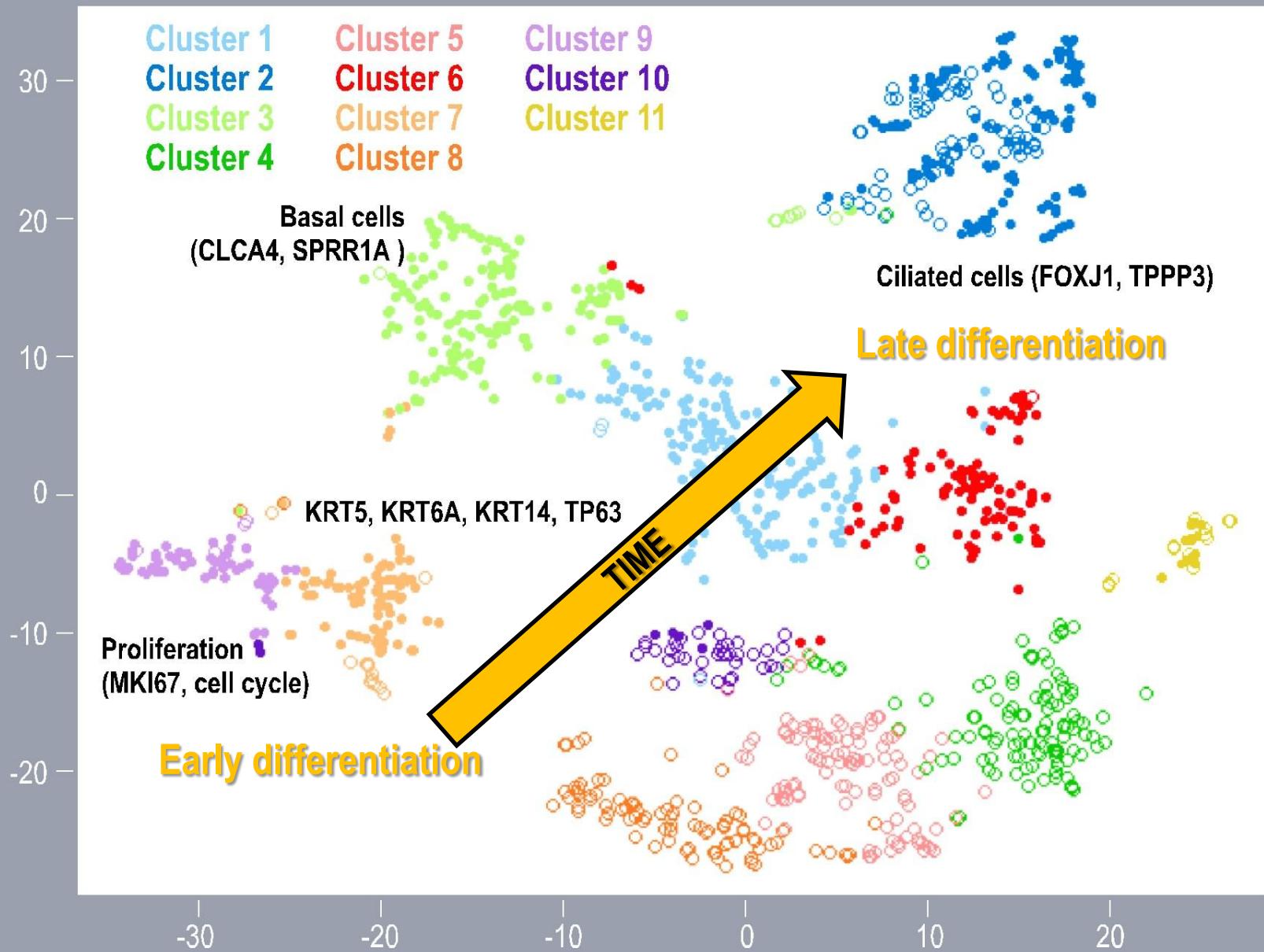


10X Chromium - million-scale single cell

1.308.421 single cell expression profiles



- the 1 million single-cells were processed as 136 libraries,
- across 17 Chromium chips, 8 samples each,
- 4 HiSeq 4000 flowcells (10 billions reads → **7.500 reads/cell**),
- this work was completed by one person in one week



lpsmc

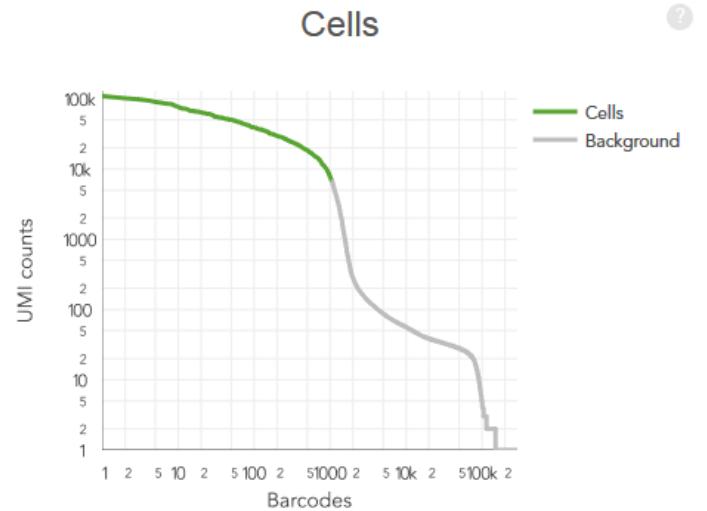
Melanoma

Estimated Number of Cells
1,067

Mean Reads per Cell
132,400 Median Genes per Cell
3,145

Sequencing

Number of Reads	141,271,047
Valid Barcodes	97.8%
Reads Mapped Confidently to Transcriptome	56.2%
Reads Mapped Confidently to Exonic Regions	60.0%
Reads Mapped Confidently to Intronic Regions	14.2%
Reads Mapped Confidently to Intergenic Regions	4.9%
Sequencing Saturation	64.2%
Q30 Bases in Barcode	93.5%
Q30 Bases in RNA Read	61.4%
Q30 Bases in Sample Index	84.8%
Q30 Bases in UMI	92.3%

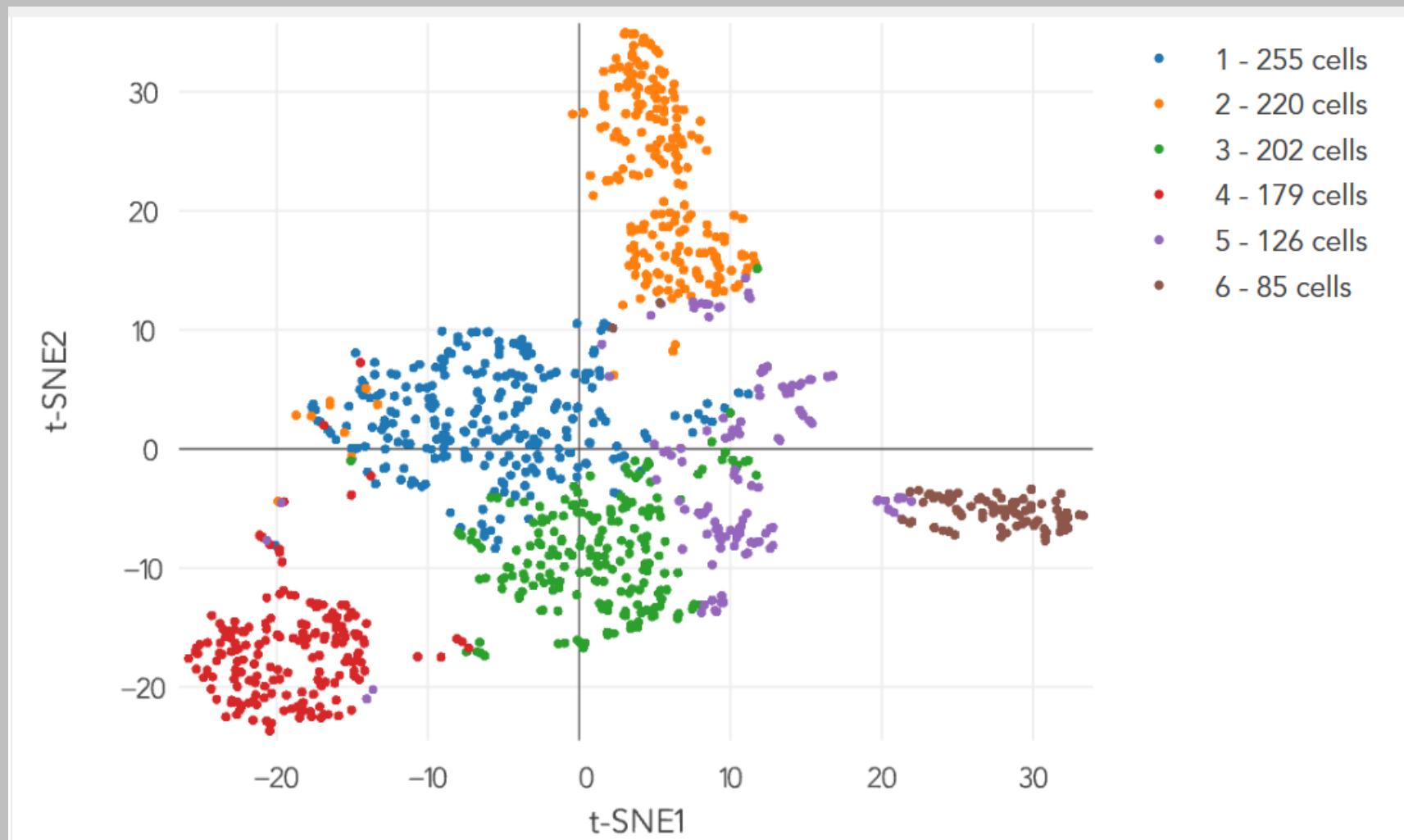


Estimated Number of Cells	1,067
Fraction Reads in Cells	82.3%
Mean Reads per Cell	132,400
Median Genes per Cell	3,145
Total Genes Detected	20,581
Median UMI Counts per Cell	17,605

Sample

Name	Melanoma_3
Description	
Transcriptome	GRCh38

Melanoma

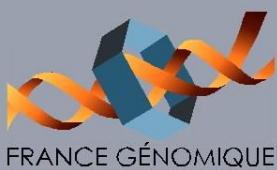


Method	SCUBA pseudotime	Wanderlust	Wishbone	SLICER	SCOUP	Waterfall	Mpath	TSCAN	Monocle	SCUBA
Visual abstract										
Structure	Linear	Linear	Single bifurcation	Branching	Branching	Linear	Branching	Linear	Branching	Branching
Robustness strategy	Principal curves	Ensemble, starting cell	Ensemble, starting cell	Starting cell	Starting population	Clustering of cells	Clustering of cells using external labeling	Clustering of cells	Differential expression	Simple model
Extra input requirements	None	Starting cell	Starting cell	Starting cell	Starting population	None	Time points	None	Time points	Time points
Unbiased	+	±	±	±	±	+	-	+	-	-
Scalability w.r.t. cells	-	-	±	±	-	±	+	+	-	±
Scalability w.r.t. genes	+	+	+	+	-	+	±	±	±	+
Code and documentation	-	±	+	±	+	±	+	+	+	±
Parameter ease-of-use	+	+	+	+	-	±	-	+	+	+

Conclusions

- Nouvelles approches expérimentales, qui vont probablement changer certaines approches médicales → Tricorder
- Nécessité d'appréhender des données d'un nouveau type (génétique, bioinformatique) qui servent dès aujourd'hui à orienter certaines pratiques médicales
- Explosion des outils « single cell » qui offrent aujourd'hui l'opportunité de capter des variations jusqu'à présent cachées dans les fluctuations du temps biologiques

Acknowledgements



Association pour la Recherche sur le Cancer



IPMC, 660 route des Lucioles, Sophia-Antipolis
06560 Valbonne France
www.ipmc.cnrs.fr

