

Enabling melanoma research with validated cell lines

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Comparing Tissue and Cell Line Banks

	Tissues	Cell Lines
Ethics	Recipient of tissue has local ethical approval	Ethical approval for cell line and specific information not required from recipient
Technology	Standardised protocols	Considerable lab-lab variation in methods & success rate
Time to prepare	Rapid	Months
Specimen Validation	Variable quality limited validation	+/- success in establishing line +/- quality (contamination, genotype, success in growing stored sample)
Applications	<u>Dead</u> (usually) Discovery Answer clinical hypotheses	<u>Live</u> Basic research Cell biology, drug discovery, cancer interactions with systems, eg immune, stem cell biology, in vivo models (xenografts)
Value	New specimens needed	Knowledge of each specimen accumulate, is shared, & can be confirmed by others
Resource	Tissue limited	∞

Cell Line

- Primary Culture: culture of cells directly from tissue
- Cell Line: “Passaged”
- Continuous cancer cell lines: Spontaneously immortalised, unlimited ability to expand



Cell Lines: Characteristics & Uses

- Unlimited supply of genetically uniform cells
- Outgrowth of more rapidly dividing cells → uniform phenotype (culture condition dependent)
- Unique features of ABN Cell Line Bank
 - Validations
 - Matched lymphoblastoid cell line
 - Limited patient data (age, sex, survival)
 - Potential for linkage of data from different researchers (e.g. gene expression studies)



Potential Uses

Research

- Immunological and expression studies
- Genetic studies – genetic mutations that contribute to tumorigenesis e.g. BRAF
- Pharmaco-genomic studies -
 - genome-wide approaches to finding which genetic/epigenetic changes in cancer cells determine their response to drugs.
- Cell biology



Cell Lines: commercial issues

- Estimated cost of establishing and banking a cell line at American Type Culture Collection - ~USD30,000*
- Material Transfer Agreement – standard
 - Originator retains ownership
 - Cell lines not to be distributed to 3rd parties
 - Not for use in humans

*Hay R. J. Banking and strain data of cell cultures. Cell Line Banking. p.224. Cell Culture Department, ATCC, 1983



Mission of Cell Bank

- storing and distributing authenticated cell lines (live or in tissue microarrays)
- provide matched tumour and genetically normal (Epstein-Barr virus-transformed lymphoblastoid) cell lines (i.e. from same patient)
- melanoma and glioblastoma



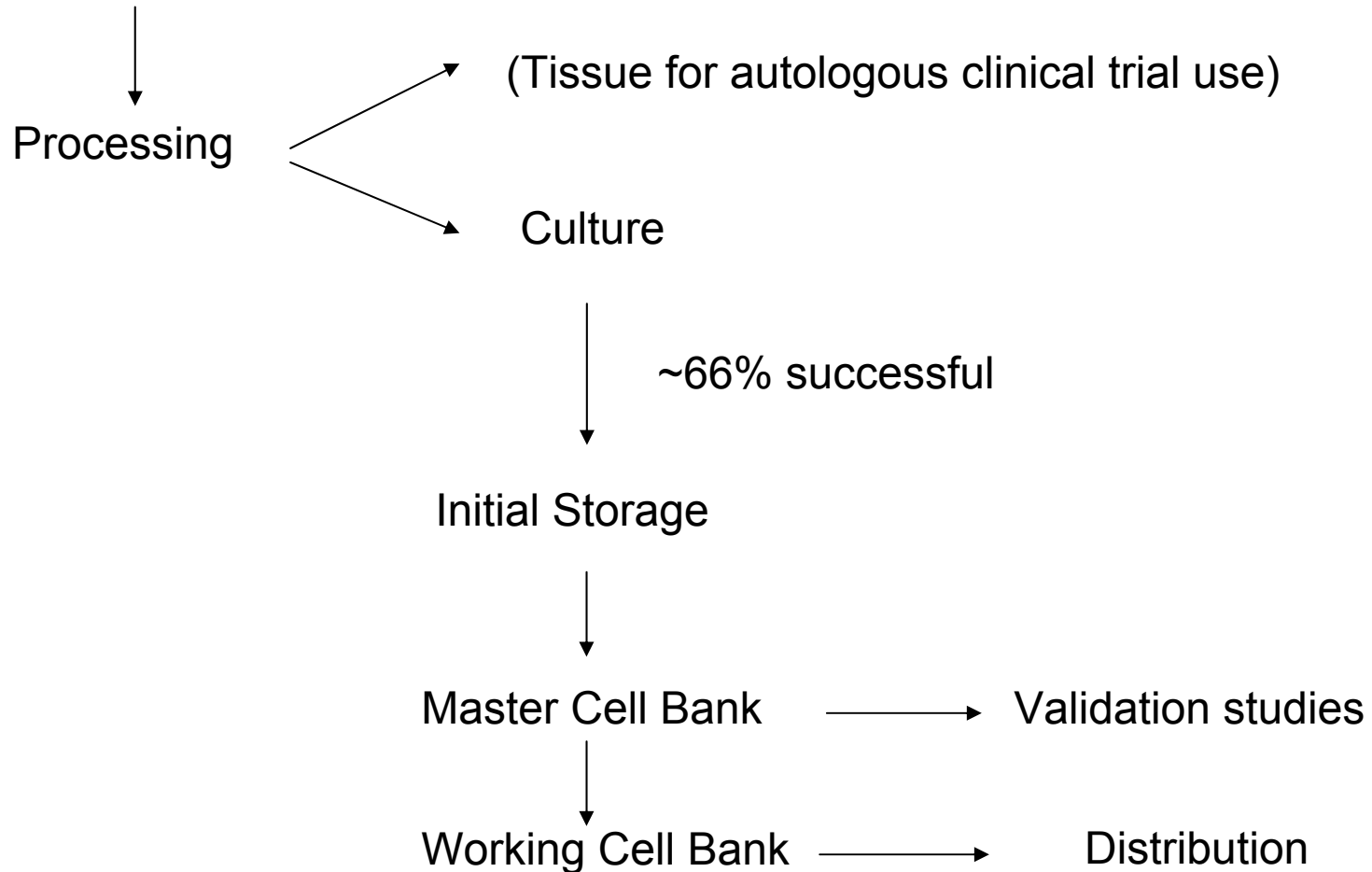
Patients

- Phase I – III clinical trials for patients with advanced melanoma or primary glioblastoma
 - ~30 Stage IV melanoma
 - ~60 Stage III melanoma
 - ~10 glioblastoma
- Tissues taken by surgery for therapeutic or clinical trial purposes
- Potential for use of tissues from non-trial patients in future



Banking Scheme

Specific Consent for Tissue Storage



Quality Control

- **Bacterial and Fungal Contamination**
 - TSB / thioglycolate broths
- **Mycoplasma**
 - PCR
 - Hoechst staining on co-cultured indicator line
- **Authentication**
 - Short tandem repeats



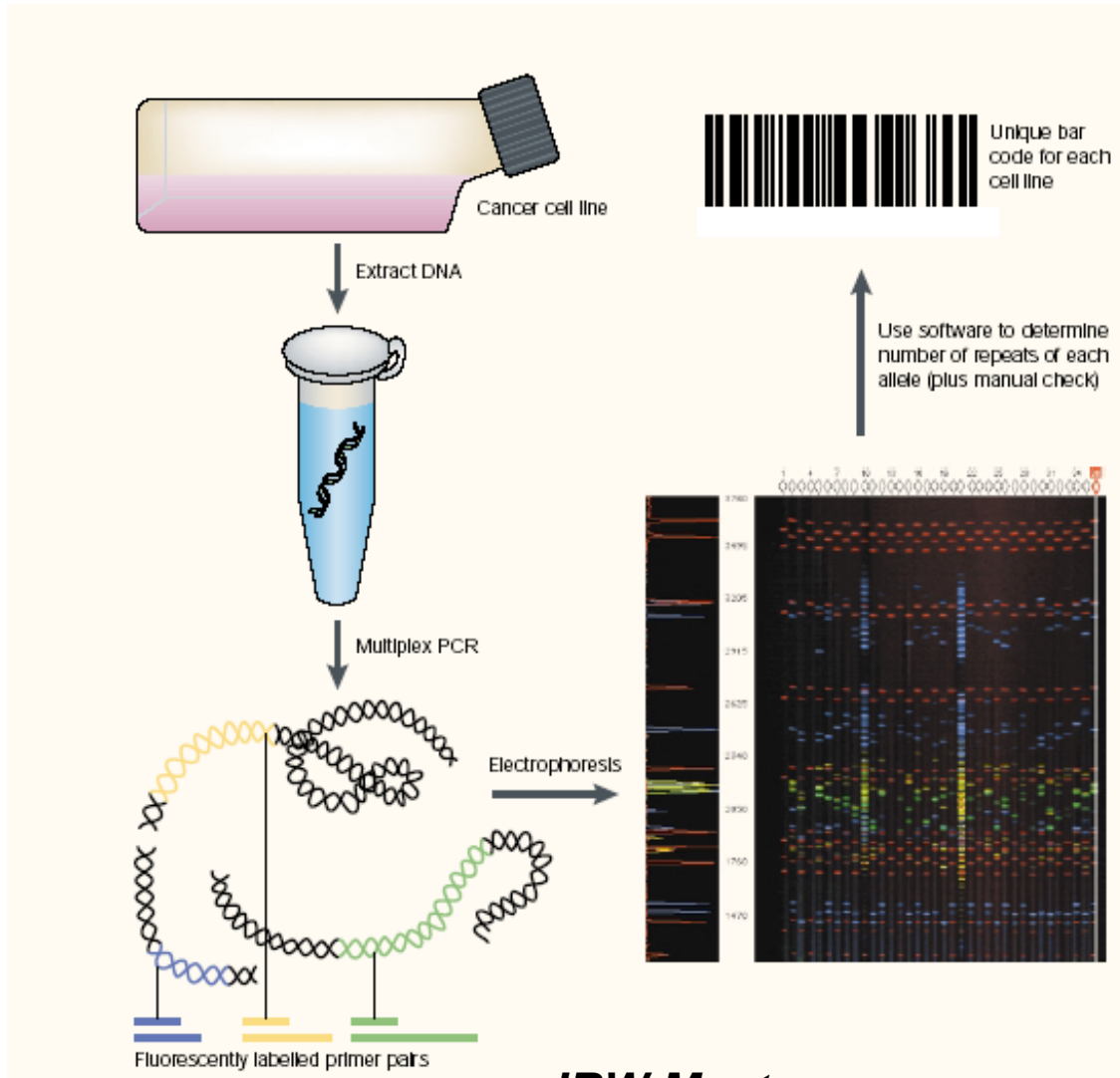
Cell line cross-contamination

- Origin
 - Poor culture technique
 - Clerical error
- Problem
 - HeLa: Walter Nelson-Rees
 - 17 – 36% of all cell lines are from a different individual or different species
 - an increasing problem!

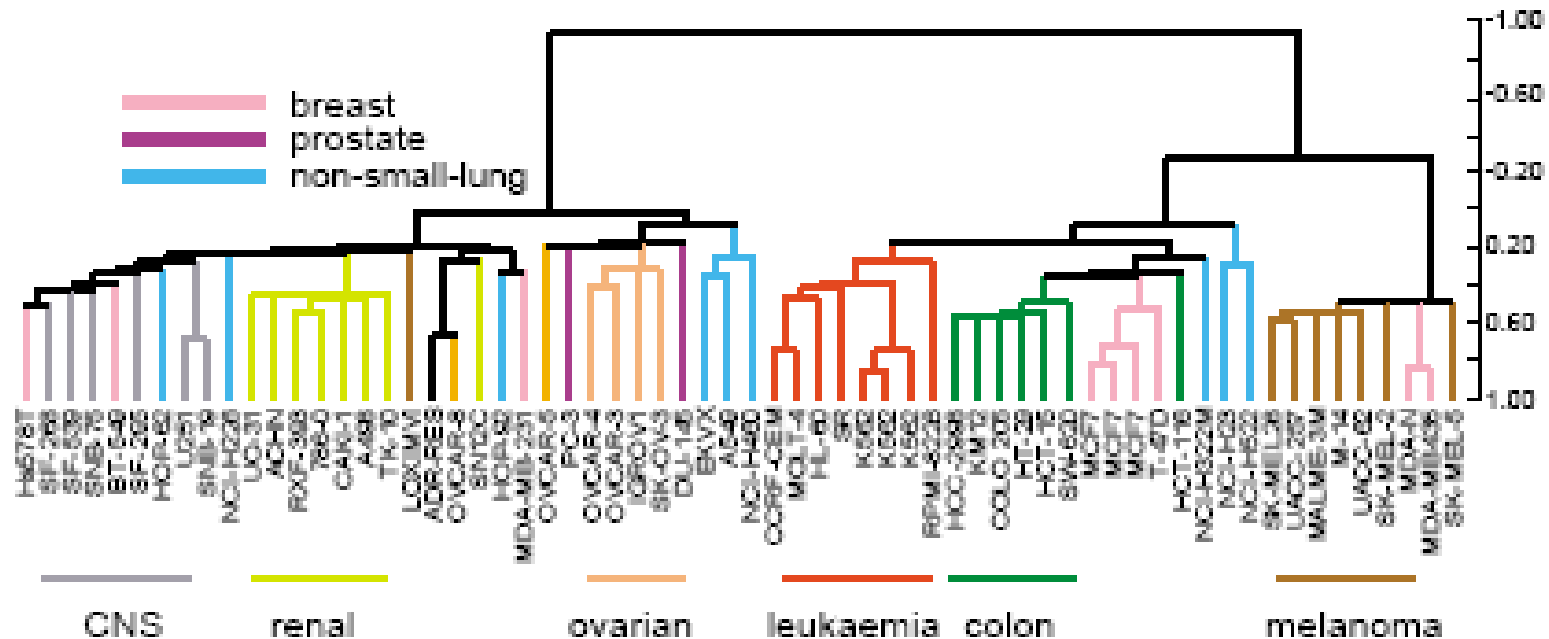


DNA profiling of short tandem repeats.

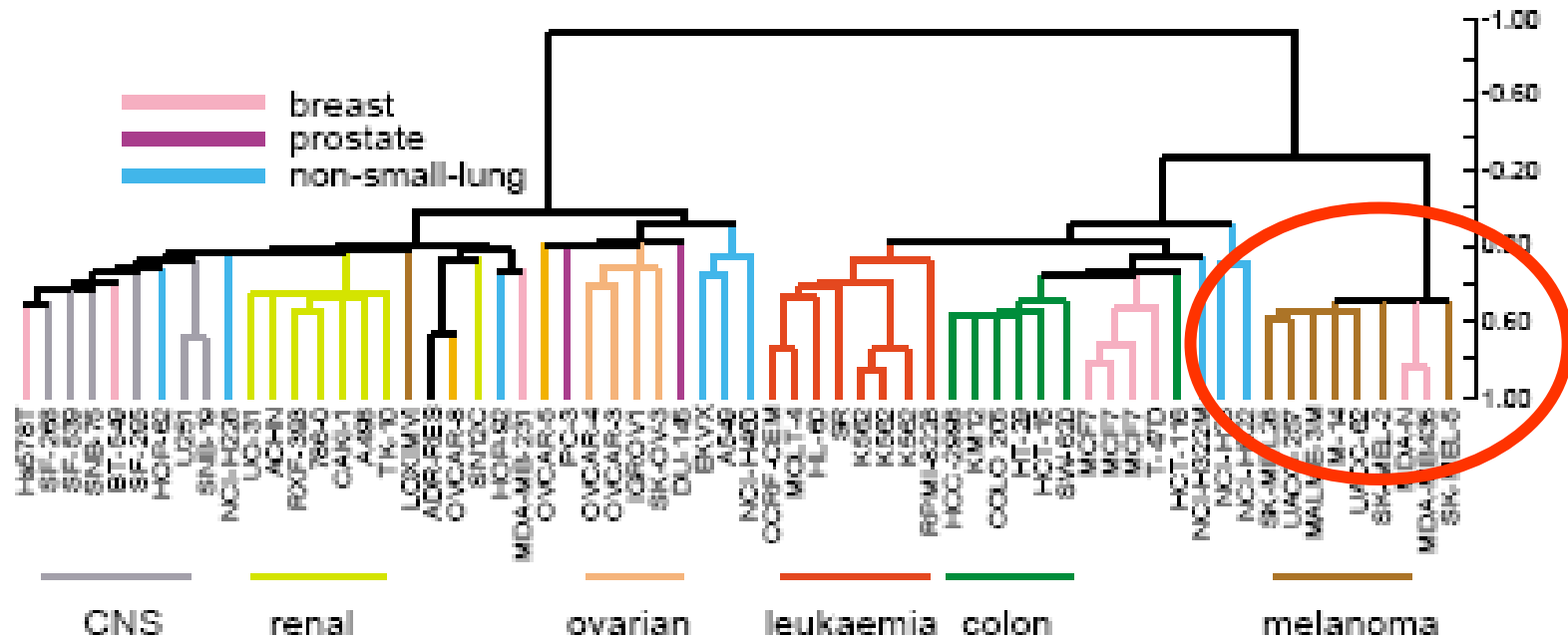
-a 'bar code' or international reference standard for cell lines



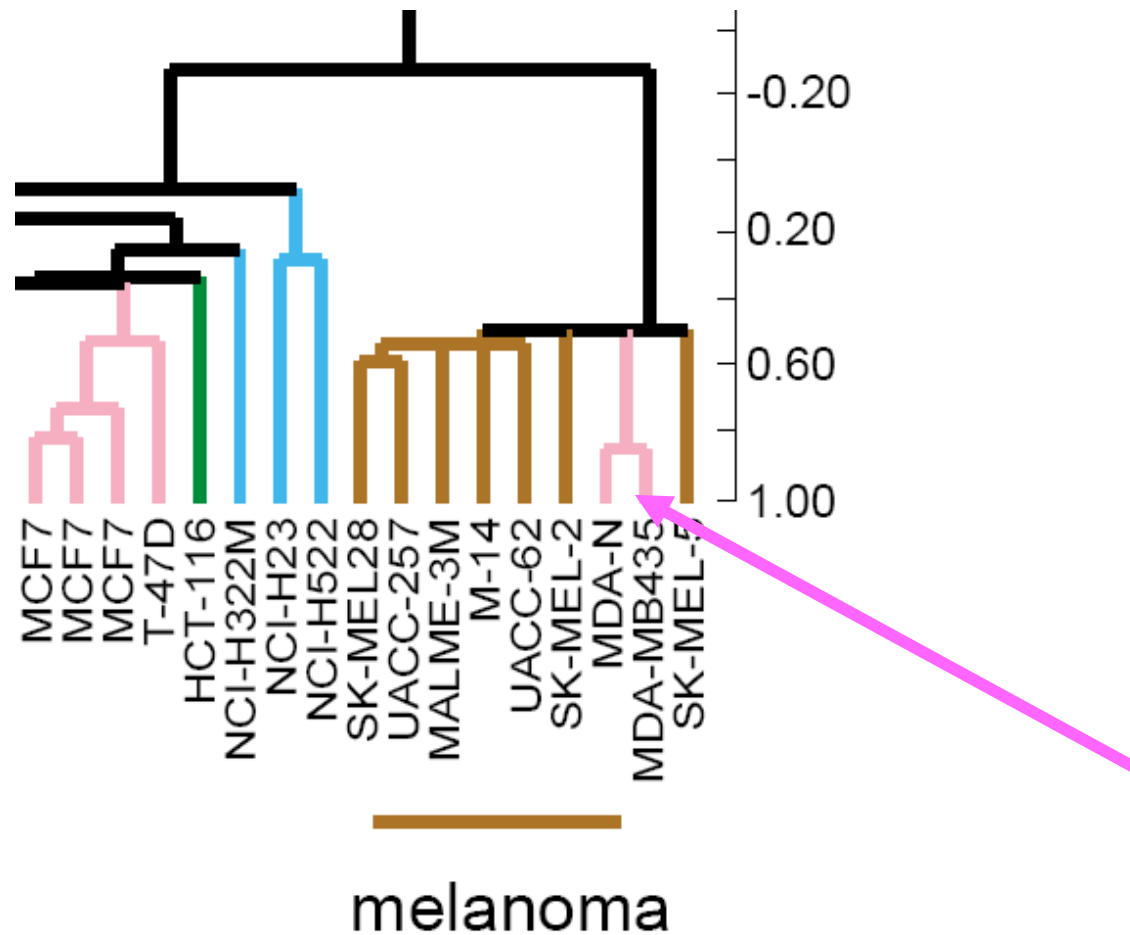
Expression Profiling Cell Lines



Expression Profiling Cell Lines



Expression Profiling Cell Lines



Progress

- Melanoma Lines: 91 (30 Stage IV, 61 Stage III) – near complete
- Matched LCL: 19 (75 when complete)
- Contamination: 83/83 tested negative
- Genotype: 23/23 tested OK vs autologous LCL and no match to any other cell line yet tested

Cell lines: uses

Cancer Antigen discovery

- Mix patient's lymphocytes + patient's melanoma cells
- Grow culture of cells that can kill patient's melanoma cells
- Define targets used by the immune system - are they mutated proteins or conserved?



Cell lines: uses

- Gene expression studies
 - Isolate RNA from melanoma cells
 - Measure amount of each individual RNA type on chip (~30,000 genes)
 - Correlate with clinical outcome

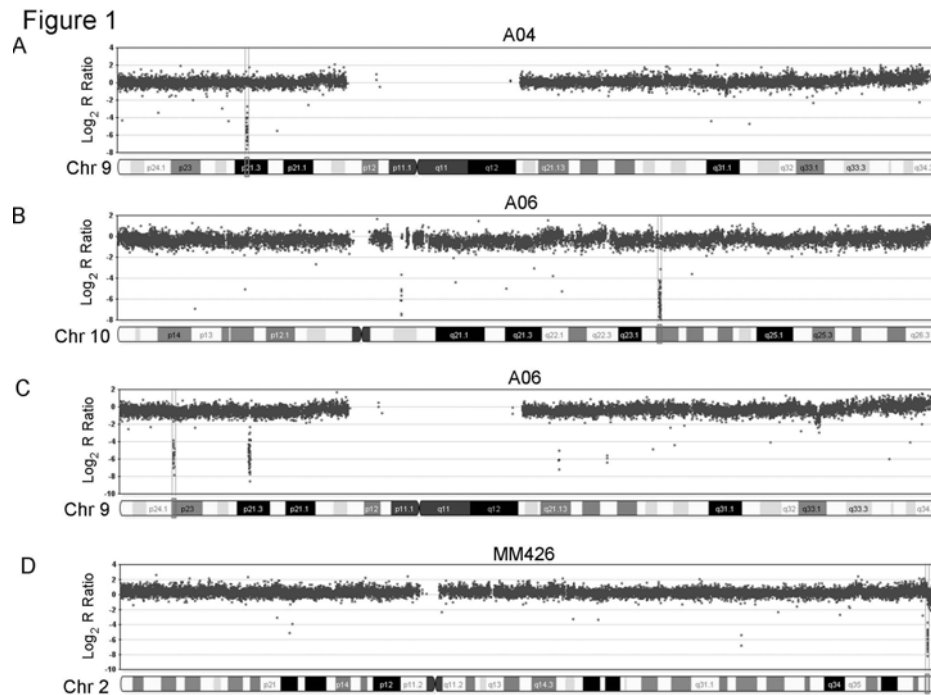


Applications: LOH

- SNP analysis

Genome-wide loss of heterozygosity and copy number analysis in melanoma using highdensity single-nucleotide polymorphism arrays

Mitchell Stark, Nicholas Hayward, Cancer Res 2007



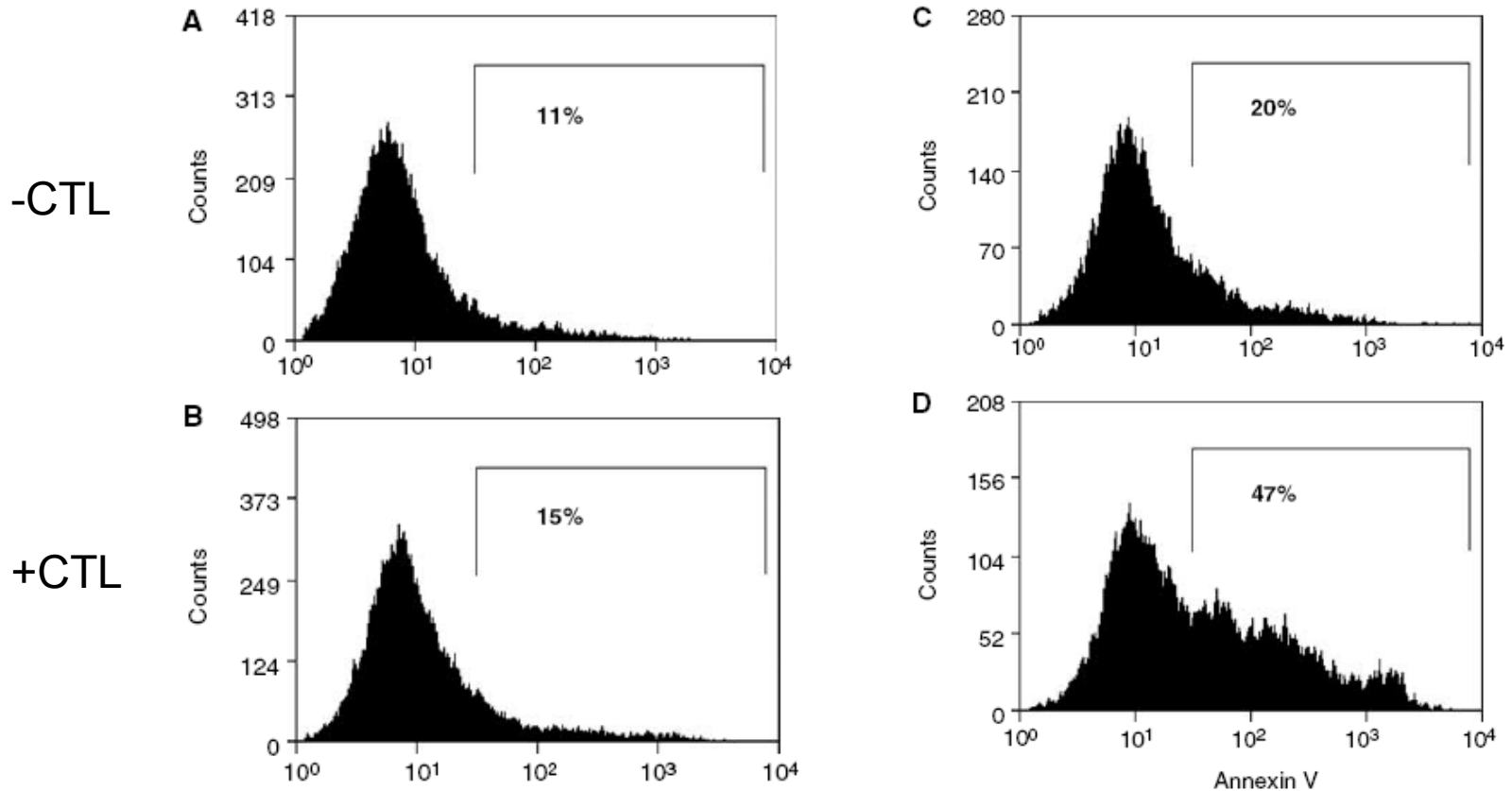
Cell biology

Drug Effects

Temozolomide and fotemustine induces apoptosis and necrosis in melanoma cells: role of MGMT and MMR in determining sensitivity

Naumann et al., under review

Drug interactions with the immune system



Melanoma line A02-M was chosen for a Bcl-2 inhibition study on the basis of high expression of Bcl-2

Commercial application

- Cell lines generated pre-ABN licensing to 2 companies

Future:

Complete genome/exome sequencing!

Summary

- Aim: supply appropriately consented, validated matched pair cell lines for research
- Current source of cell lines: patients enrolled in immunotherapy trials
- Potential: a rich source of correlative data

And to the other ABN members..

- **Thanks!**