Identifying microRNA signatures of chemo-resistance in colorectal cancer

Presented by:

Team Miscreant (MicroRNA Screening and Targeting)

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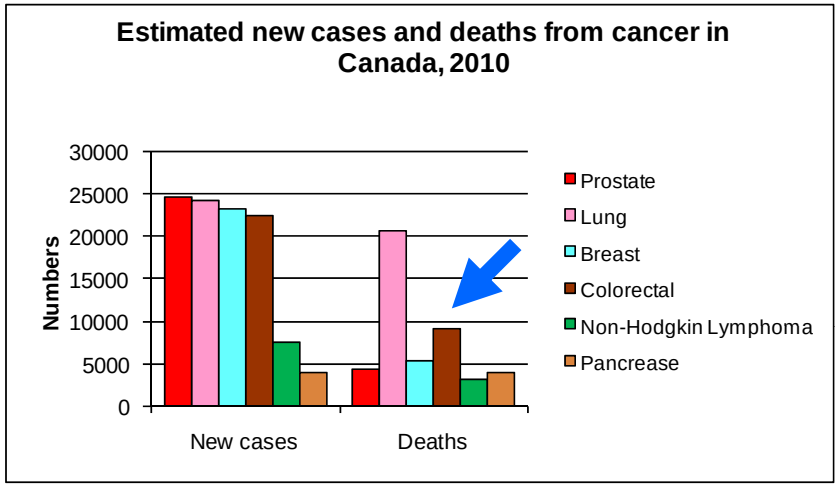
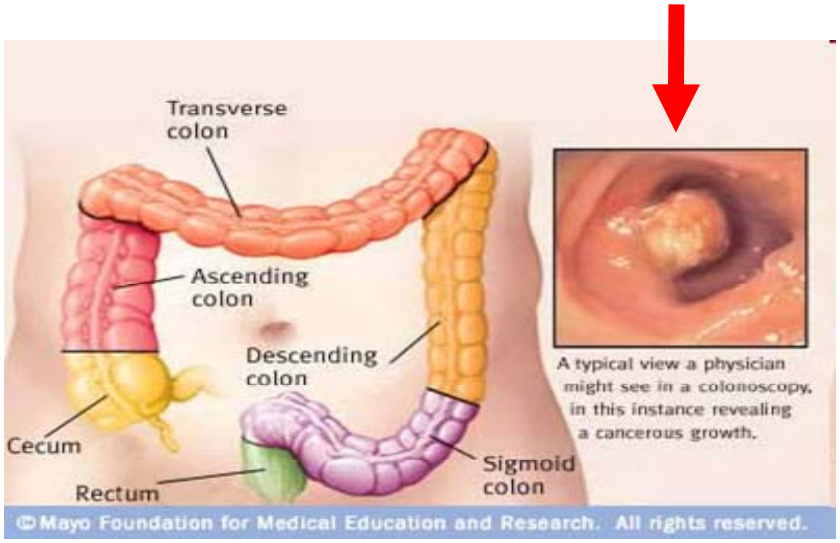
Colorectal Cancer (CRC): the second most deadly form of cancer in Canada

- Fourth most common form of cancer worldwide
- 22,500 Canadians will be diagnosed with CRC and 9,100 will die from it in 2010
- Cancerous growth in the colon, and rectum
- Originate from benign adenomas (polyps) in the normal colonic mucosa, through accumulation of genetic abnormalities

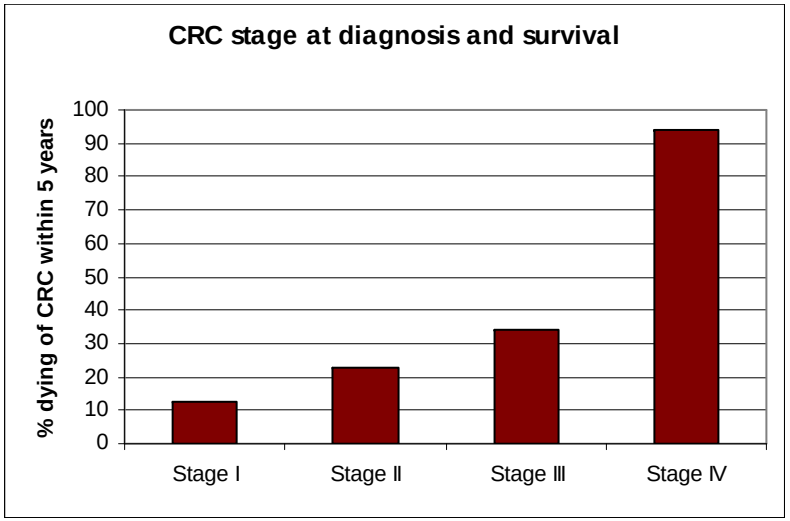


Source: Canadian Cancer Statistics, 2010



CRC is highly treatable if detected early

- CRCs have a recognizable early stage
- Patients diagnosed at an early stage have a survival rate of over 90%



Maroun et al., 2003, CDIC Vol. 24 No. 4, 1-17

- 50% of patients will succumb to their disease and 90-100% of the terminal cases are metastatic
- Surgery is a major treatment modality for primary CRC and some liver metastasis
- Adjuvant chemotherapy is recommended for Stage III and some high risk stage II colon cancer (BC Cancer Agency).

Chemo-resistance in CRC

BCCA recommendation on chemotherapy regimens of CRC:

Oxaliplatin/5FU/ Leucovorin regimen, UGIAJFFOX for patients with resected node positive (Stage 3) colon cancer

- Drug resistance is thought to cause treatment failure in over 90% of patients with metastatic cancer (Longley et al., Biochim Biophys Acta. 2006 Dec;1766(2):184-96).
- Response rates for 5-FU as a single first-line treatment in advanced CRC are only 10–15% (Johnston et al., *Anti-cancer Drugs* **12** (2001), pp. 639–646)
- Combining 5-FU with the newer chemotherapies irinotecan (CPT-11) and oxaliplatin has improved response rates for advanced CRC to 40–50% (Giacchetti et al., *J. Clin. Oncol.* **18** (2000), pp. 136–147; Douillard et al., *Lancet* **355** (2000), pp. 1041–1047)

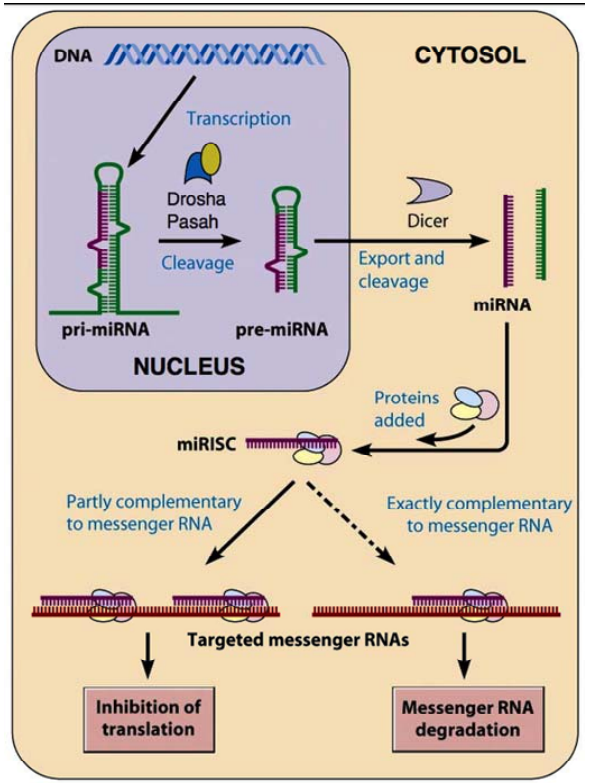
Possible reasons why robust molecular predictors of response to treatment in CRC are lacking:

- **Intrinsic** resistance represents **minor/negligible** population of cells that are “diluted” out by the rest
- Resistance is predominantly **acquired**
- Discovery **methods** employed are not suitable
  -bias, sensitivity, dynamic range etc
- Biomolecules investigated may **NOT be great classifiers**

Can miRNAs be used as predictors of chemoresistance in CRC?

miRNA and chemoresistance in cancer

- Small non-coding RNAs (~22 nt)
- Negative regulators of mRNA expression and translation



http://www.highlighthealth.com/resources/micrornas-in-human-health-and-disease/

MicroRNA expression profiles of a panel of 60 diverse human cancer cell lines (NCI-60) showed significant correlations with the potency patterns of the 3089 chemical compounds, suggesting their role in chemoresistance.

Blower et al., Mol Cancer Ther. 2007 May;6(5):1483-91.

miRNA and colorectal cancer

Deregulated expression of miRNAs in CRC tissues compared with normal tissues

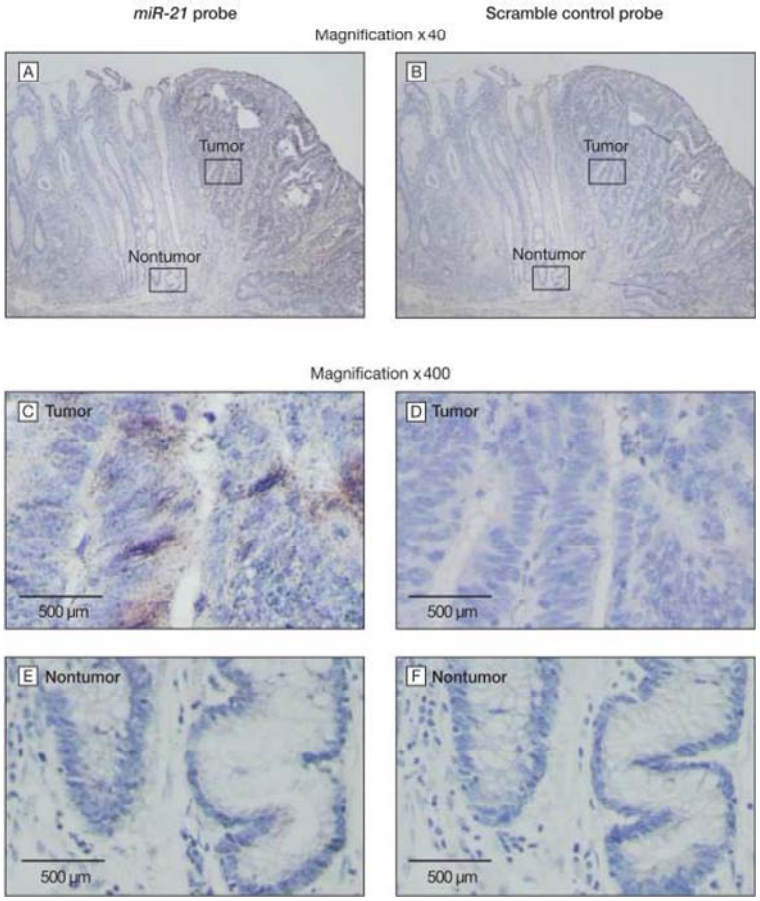
Up-regulated in CRC	Down-regulated in CRC
miR-15b	miR-9
miR-17-3p	miR-30-3p
miR-17-5p	miR-101
miR-18a	miR-122
miR-19a	miR-124a
miR-20a	miR-126
miR-21	miR-129
miR-31	miR-133b
miR-92	miR-137
miR-96	miR-143
miR-133b	miR-145
miR-135b	miR-328
miR-181b	miR-451
miR-183	
miR-191	
miR-200c	

miRNAs associated with advanced TNM stage and shorter survival in CRC

Advanced TNM stage	Up-regulated	miR-9 miR-21	miR-31 miR-129
Shorter survival	Up-regulated	miR-200c	miR-21
	Down-regulated	miR-320	miR-498

Liu et al., J Genet Genomics. 2010 Jun;37(6):347-58

In Situ Hybridization of *miR-21* in Colon Tumors



Schetter et al., JAMA. 2008 Jan 30;299(4):425-36.

miRNA and chemoresistance in colorectal cancer

- MicroRNA-21 induces resistance to 5-fluorouracil by down-regulating human DNA MutS homolog2 (hMSH2) (Valeri et al., Proc Natl Acad Sci U S A. 2010)
- Dysregulation of microRNA-34a expression causes drug-resistance to 5-FU in human colon cancer DLD-1 cells (Akao et al., Cancer Lett. 2010)
- miR-192/miR-215 influence 5-fluorouracil resistance through cell cycle-mediated mechanisms complementary to its post-transcriptional thymidilate synthase regulation (Boni et al. Mol Cancer Ther. 2010 Aug;9(8):2265-75)

Hypothesis: miRNA profiles differ between combination chemo-resistant and sensitive CRC tumours.

Overall Objective: To identify miRNA signatures of chemo-resistance in CRC.

Specific aims:

- *To identify potential intrinsic chemo-resistance associated miRNA signatures (retrospective).*
- *To validate any signatures identified in aim 1 in an independent prospective 'validation' set.*
- *To enrich a cell population with a miRNA signature that may be inherently associated with chemo-resistance.*

Aim 1: identify potential intrinsic chemo-resistance associated miRNA signatures

Retrospective clinical study

100 primary CRC tumour

- **samples prior to drug treatment**
- **50 responders and 50 non-responders to 5-FU based chemotherapy (UGIAJFFOX).**

- BC Cancer Agency
- Tumour Tissue Repository
- Ontario Tumour Bank

miRNA-seq

- **Illumina HiSeq2000**
- **96-well plate-based library construction protocol**
- **256 miRNA libraries to be loaded in a single run**

Analysis of miRNA profiles of responders vs non-responders

Any miRNA signatures?

Responders/non-responders: 5-YEAR disease-free survival and other endpoints (e.g. development of liver met, shrinkage of liver met, etc) will be used as a criterion

Aim 2: Validate miRNA signatures in an independent prospective 'validation' set.

Prospective clinical study

200 primary CRC tumour

- **Immediately after surgery**
- **patient never treated**
- **Collection for 2 yrs**
- **Clinical followup for 5 yrs**

Collaboration with GI group of BC Cancer Agency

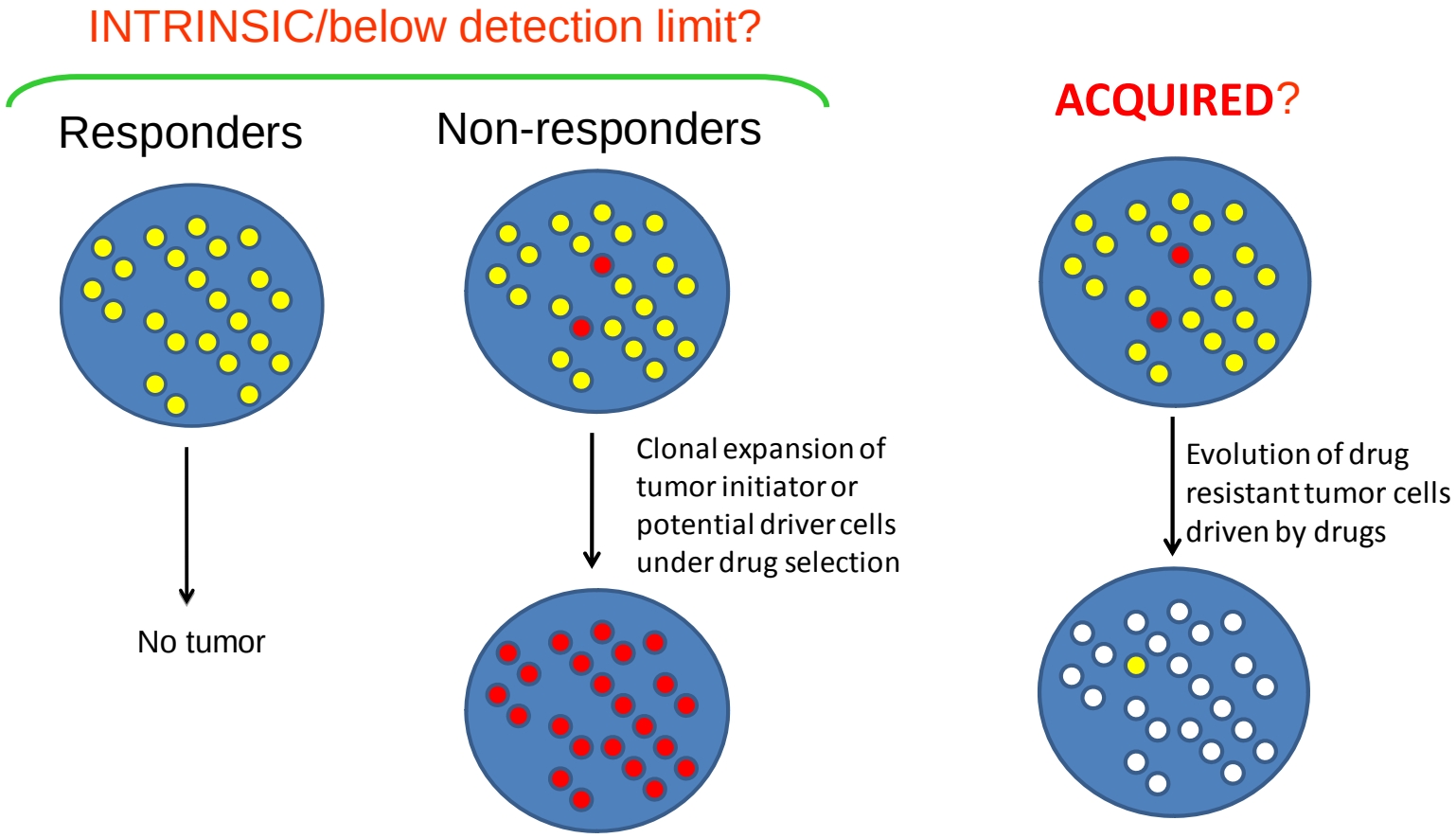
- **miRNA-sequencing and analysis as in Aim 1**
- **Validation using a different platform (NanoString)**

Analysis of miRNA profiles in relation to clinical followup data: responders vs non-responders

Any miRNA signatures?

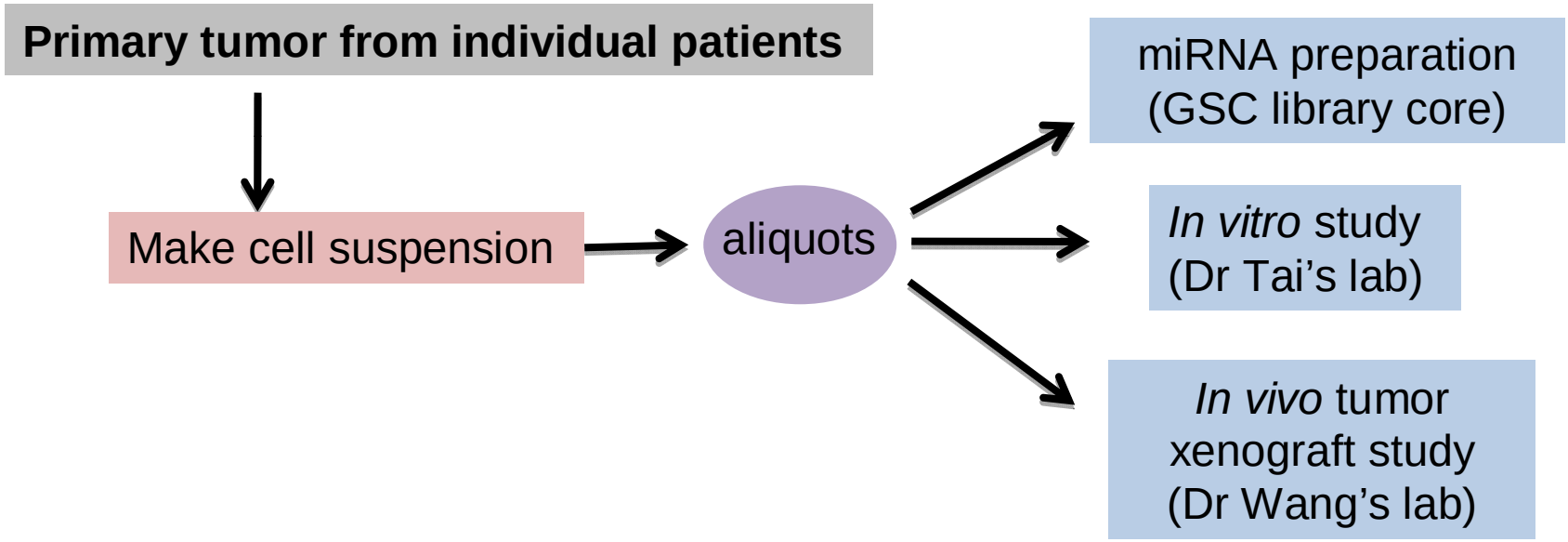
Responders/non-responders: 5-YEAR disease-free survival and other endpoints (e.g. development of liver met, shrinkage of liver met, etc) will be used as a criterion

Aim 3: Enrich a cell population with a miRNA signature that may be inherently associated with chemo-resistance.

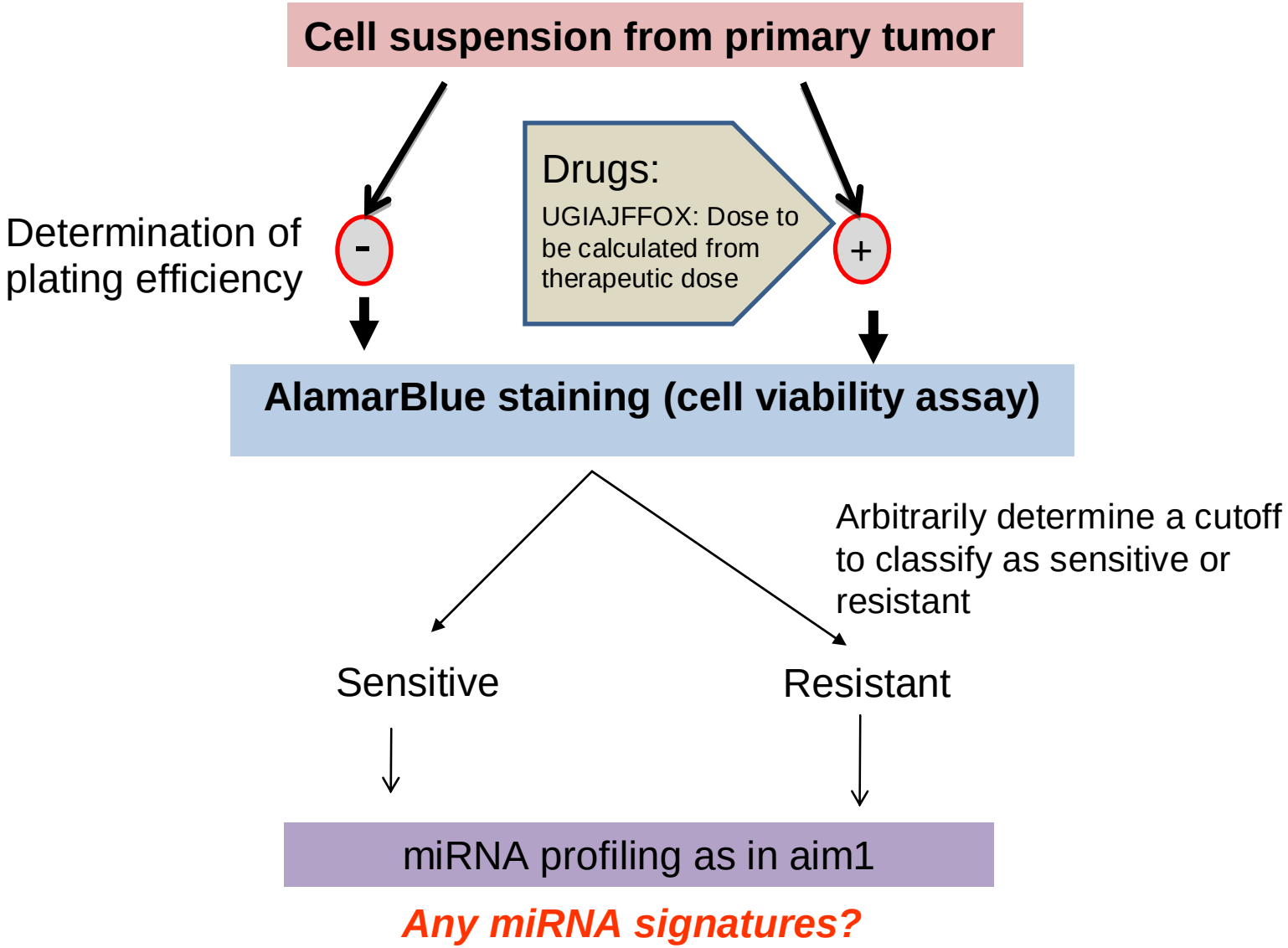


***OR combination of the two?**

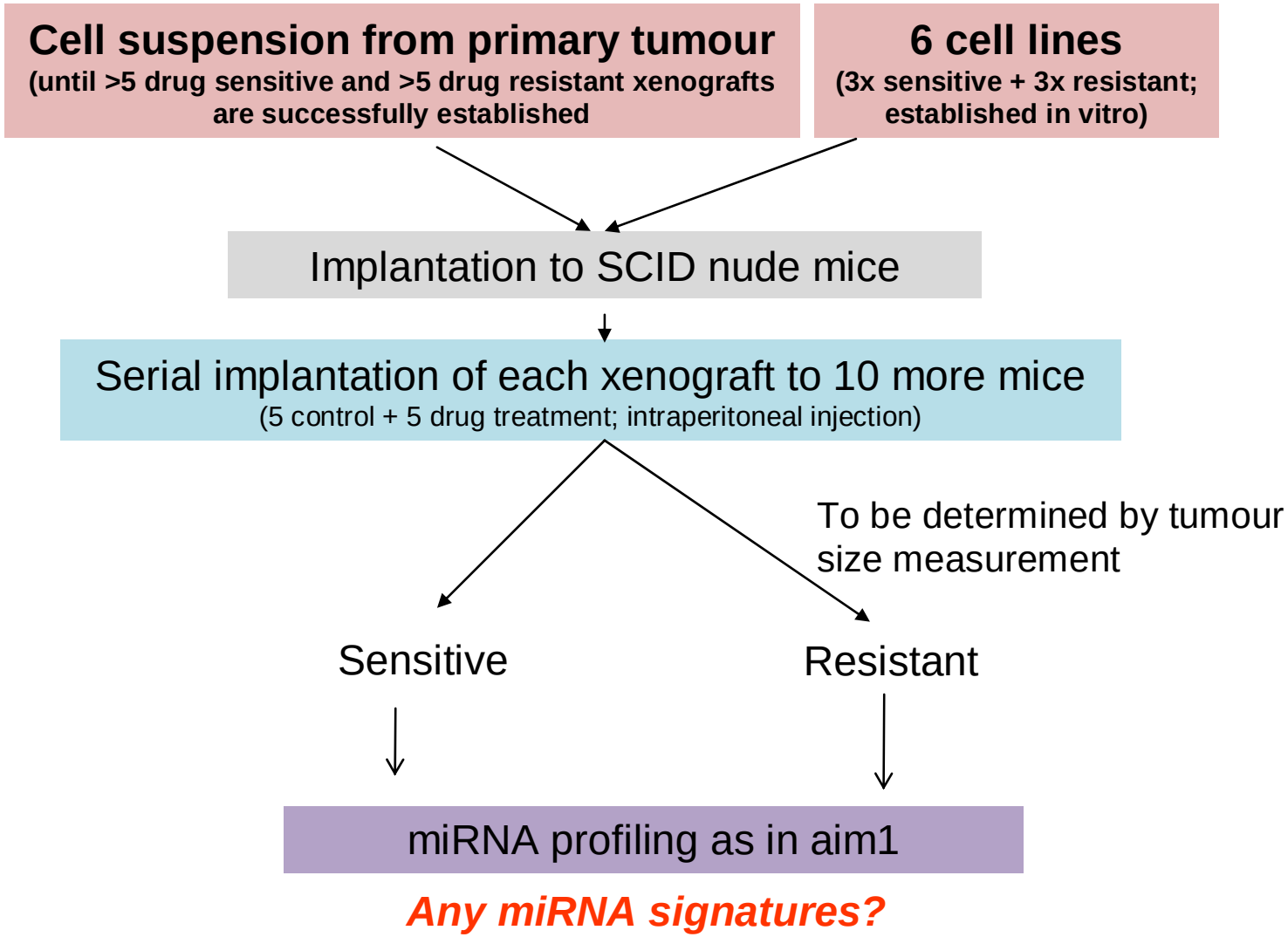
SCHEME OF PRIMARY TUMOUR PROCESSING



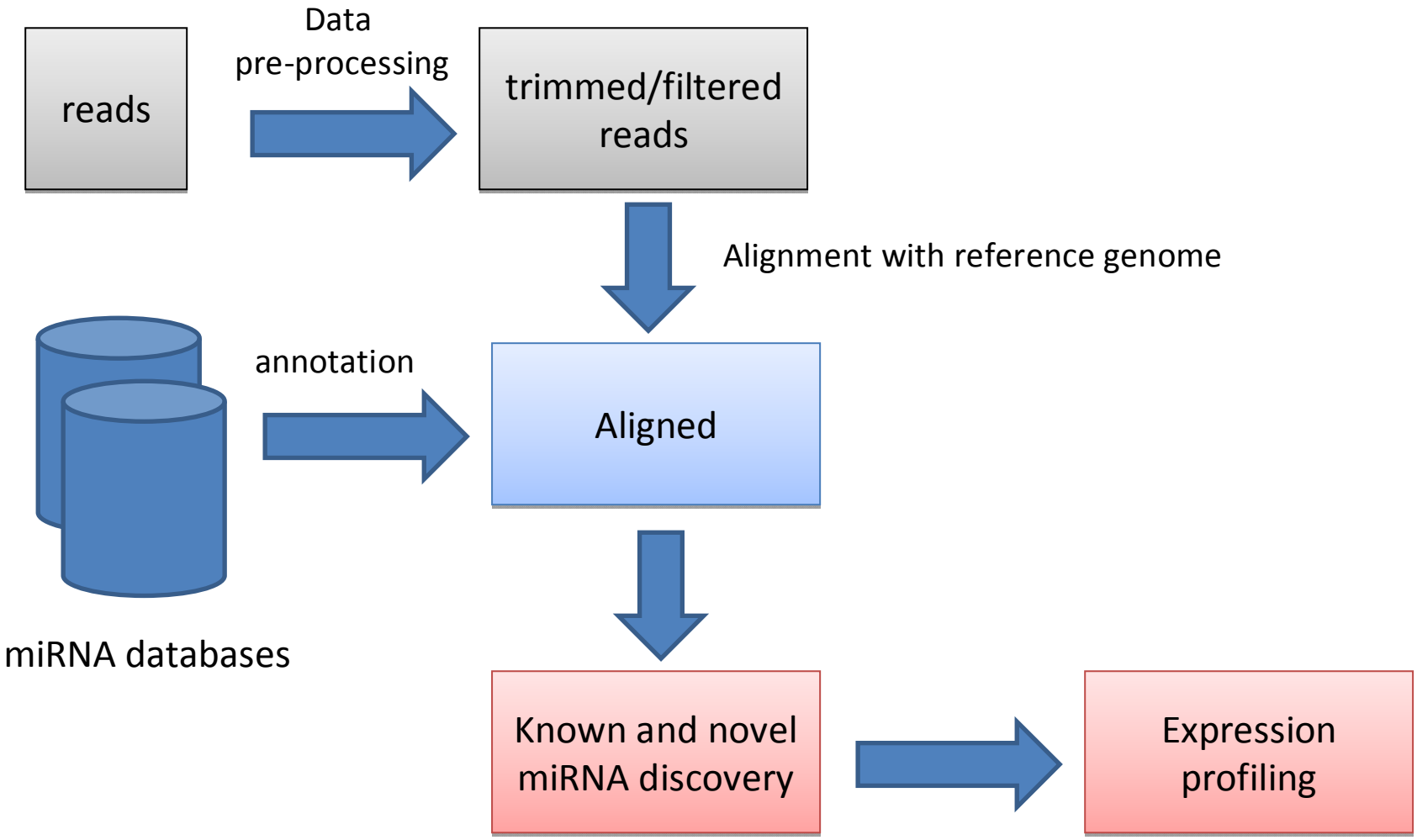
IN VITRO ENRICHMENT OF DRUG RESISTANT CELLS IN PRIMARY TUMORS



miRNA PROFILING OF CRC XENOGRAFTS AFTER DRUG TREATMENT



Analysis using TCGA miRNA discovery pipeline



Modified from Chu A. *et al*. 2010

Major Comparisons

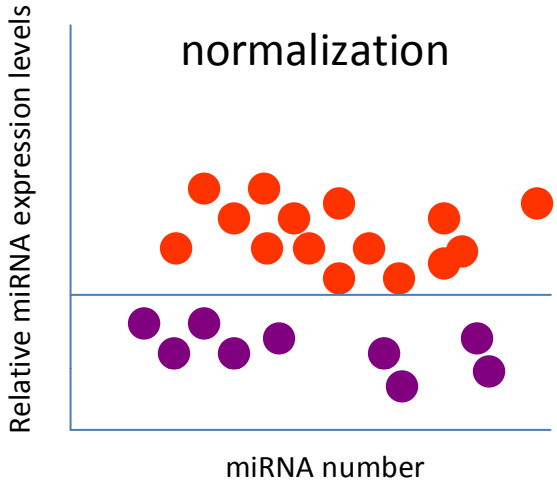
Responders Vs Non-responders (primary tumours "*in situ*")

Sensitive Vs Resistant (Primary tumours following *in vitro* enrichment in the presence of drugs)

Sensitive Vs Resistant (Primary tumours following xenograft passaging in the presence of drugs)

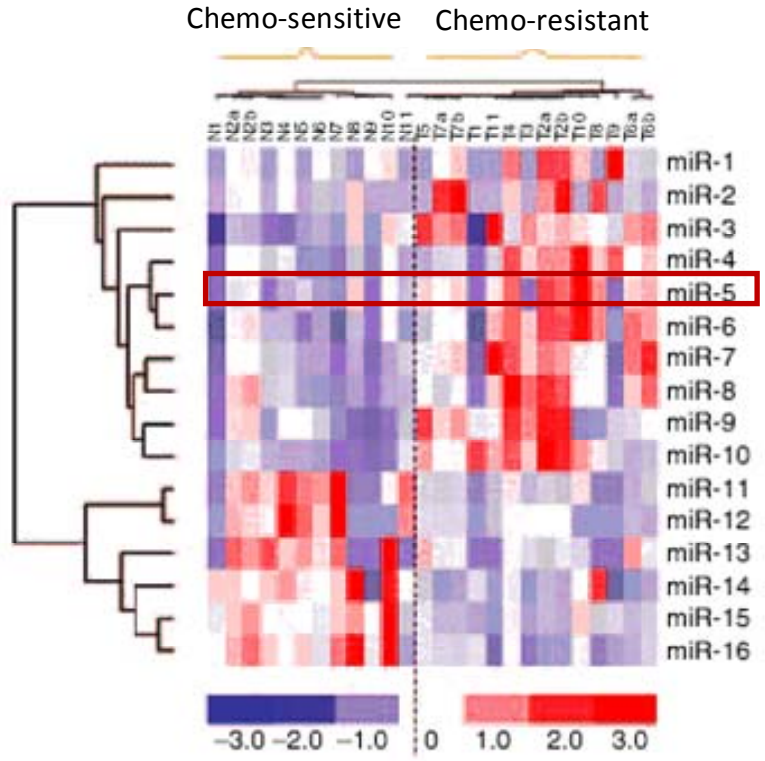
Signature-to-signature comparisons

miRNA expression profile analysis



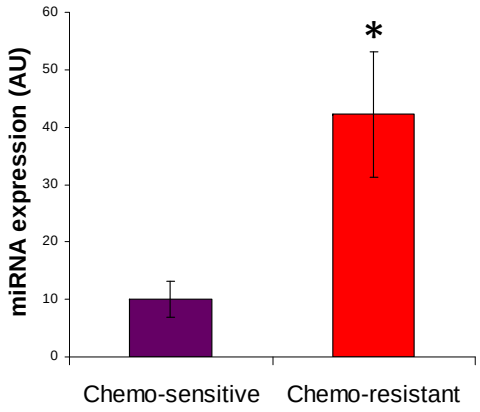
Hierarchical clustering

Derive differential expression signatures



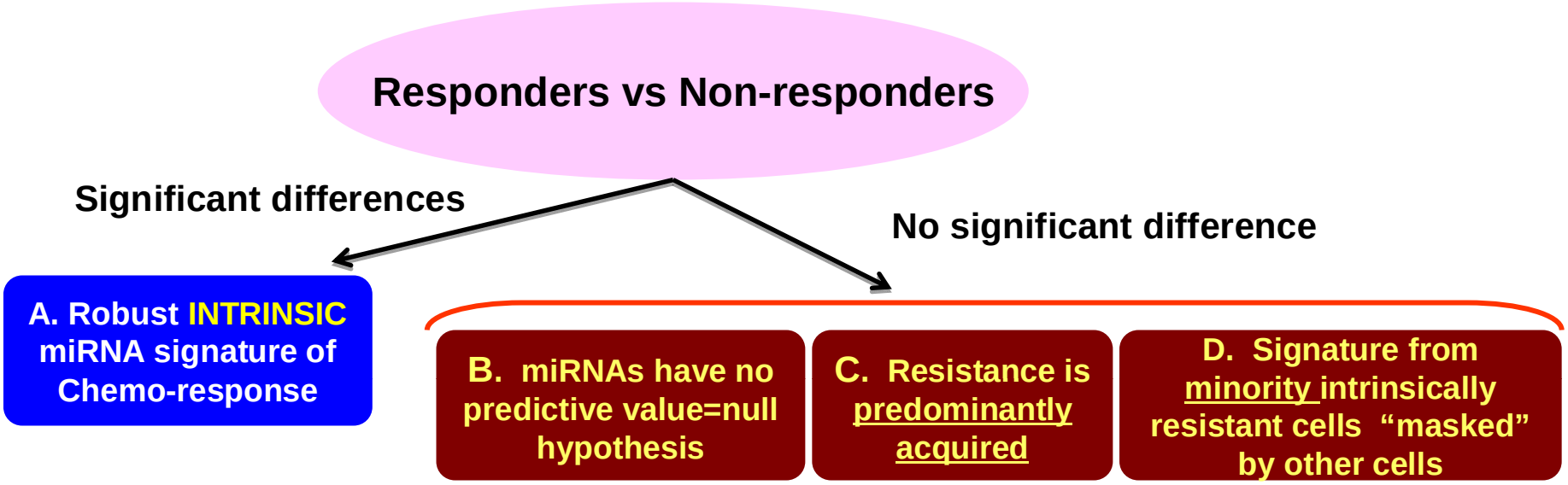
Cross sample profile-profile comparison

miR-5 expression



* Test for significance using two-sided t-test

Possible Scenarios



- ***IF (C) or (D): the primary tumour-in vitro/xenograft combo might be ideal for prediction of chemo-response***
- ***IF (B): change name to: miRNA (Masters of Immensely Rubbish and Non-sense Academics)!!!!***
- ***IF (A): change name to: SWEET! (Small RNAs do Wonders for Efficiently Enlightening Therapeutic decisions)!!!***

Significance

Exclude non-responders from treatment that does not work for them:

- spare patients poorer quality of life due to treatment
- save money for ineffective treatment
- consider alternative combination

Some of the predictor miRNAs are likely to regulate drivers of resistance

- hopefully lead to design of newer drugs

Acknowledgements

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Definitely NOT miscreants

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