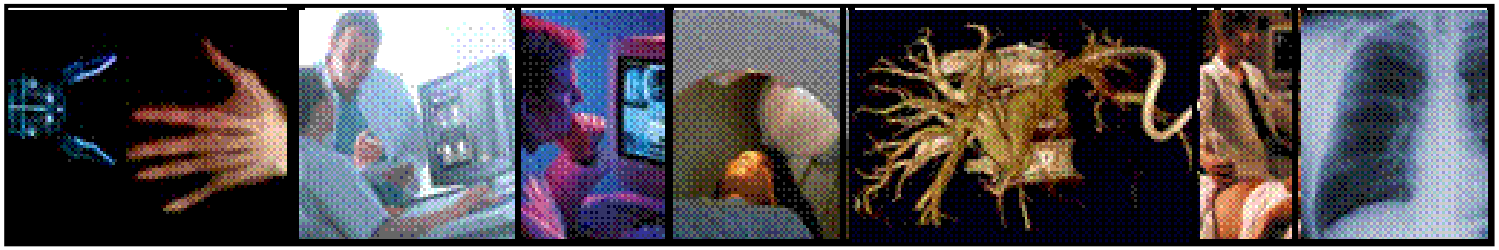

Biomarkers in Oncology: Research & Early Development

Hans Winkler
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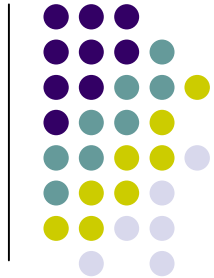
The Reality of Targeted Therapy



- In any particular indication response rates can be below 20%
- This can lead to many patients being treated without benefit
  - Subsets due to molecular heterogeneity of tumors
- Moreover, this results in the requirement for large numbers of patients to demonstrate clinical benefit and non-inferiority
  - Higher risk and cost, higher chance of failure

Cancer Biomarkers in Clinical Use

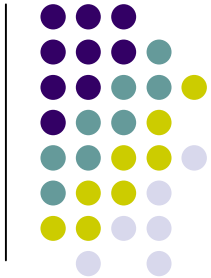
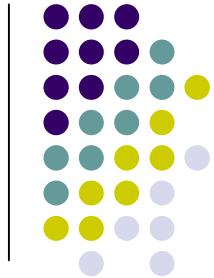


Table 1 | **US Food and Drug Administration-approved cancer biomarkers**

Biomarker	Type	Source	Cancer type	Clinical use
α-Fetoprotein	Glycoprotein	Serum	Nonseminomatous testicular	Staging
Human chorionic gonadotropin-β	Glycoprotein	Serum	Testicular	Staging
CA19-9	Carbohydrate	Serum	Pancreatic	Monitoring
CA125	Glycoprotein	Serum	Ovarian	Monitoring
Pap smear	Cervical smear	Cervix	Cervical	Screening
CEA	Protein	Serum	Colon	Monitoring
Epidermal growth factor receptor	Protein	Colon	Colon	Selection of therapy
KIT	Protein (IHC)	Gastrointestinal tumour	GIST	Diagnosis and selection of therapy
Thyroglobulin	Protein	Serum	Thyroid	Monitoring
PSA (total)	Protein	Serum	Prostate	Screening and monitoring
PSA (complex)	Protein	Serum	Prostate	Screening and monitoring
PSA (free PSA %)	Protein	Serum	Prostate	Benign prostatic hyperplasia versus cancer diagnosis
CA15-3	Glycoprotein	Serum	Breast	Monitoring
CA27-29	Glycoprotein	Serum	Breast	Monitoring
Cytokeratins	Protein (IHC)	Breast tumour	Breast	Prognosis
Oestrogen receptor and progesterone receptor	Protein (IHC)	Breast tumour	Breast	Selection for hormonal therapy
HER2/NEU	Protein (IHC)	Breast tumour	Breast	Prognosis and selection of therapy
HER2/NEU	Protein	Serum	Breast	Monitoring
HER2/NEU	DNA (FISH)	Breast tumour	Breast	Prognosis and selection of therapy
Chromosomes 3, 7, 9 and 17	DNA (FISH)	Urine	Bladder	Screening and monitoring
NMP22	Protein	Urine	Bladder	Screening and monitoring
Fibrin/FDP	Protein	Urine	Bladder	Monitoring
BTA	Protein	Urine	Bladder	Monitoring
High molecular weight CEA and mucin	Protein (Immunofluorescence)	Urine	Bladder	Monitoring

BTA, bladder tumour-associated antigen; CA, cancer antigen; CEA, carcinoembryonic antigen; FDP, fibrin degradation protein; FISH, fluorescent *in-situ* hybridization; GIST, gastrointestinal stromal tumour; IHC, immunohistochemistry; NMP22, nuclear matrix protein 22; PSA, prostate-specific antigen.

Concept & Approach

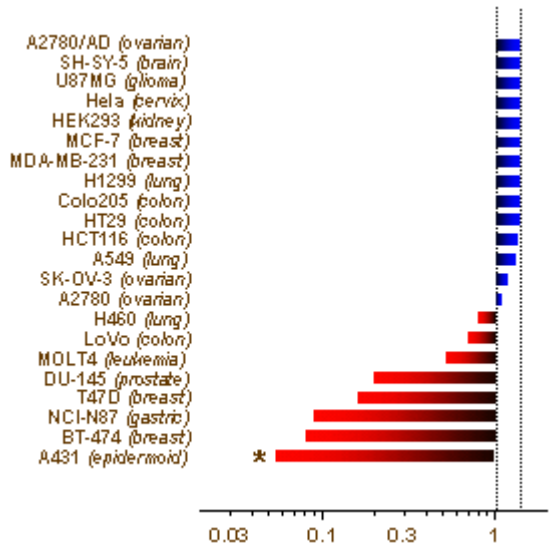


- A set of analytes (response signature) as the measure of sensitivity of a tumor to a given treatment
- Proposed Approach
  1. Identify analytes which differentiate a responding tumor cell line or *ex vivo* tumor culture from a non-responding tumor cell line or *ex vivo* tumor culture based on IC_{50}
  2. Confirm and refine the signature by data generated from primary tumors as well as external data
  3. Assess the validity of the signature in Phase 2 trials and adjust it further as necessary

Current Strategies

Prognostic signature identification

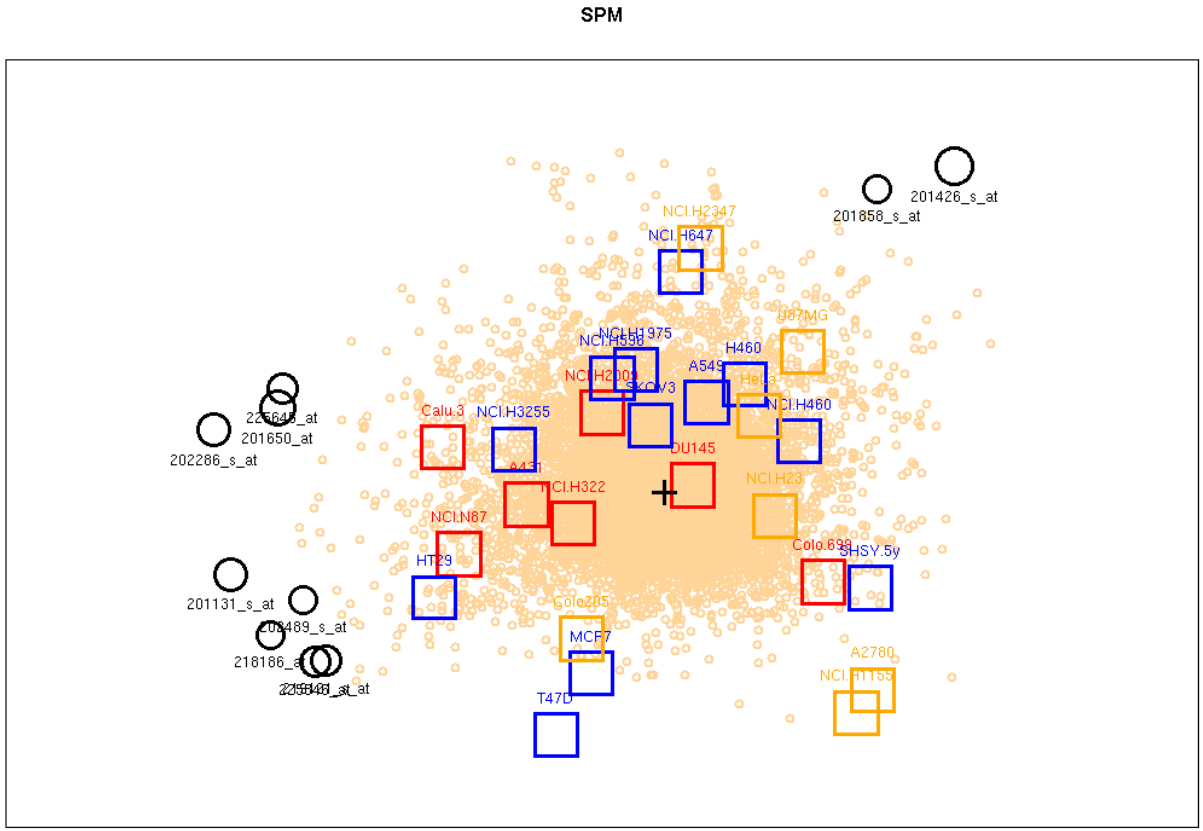
- *Identification array signature that predicts sensitivity to our candidate drugs in tumour cell lines* in vitro *before treatment*



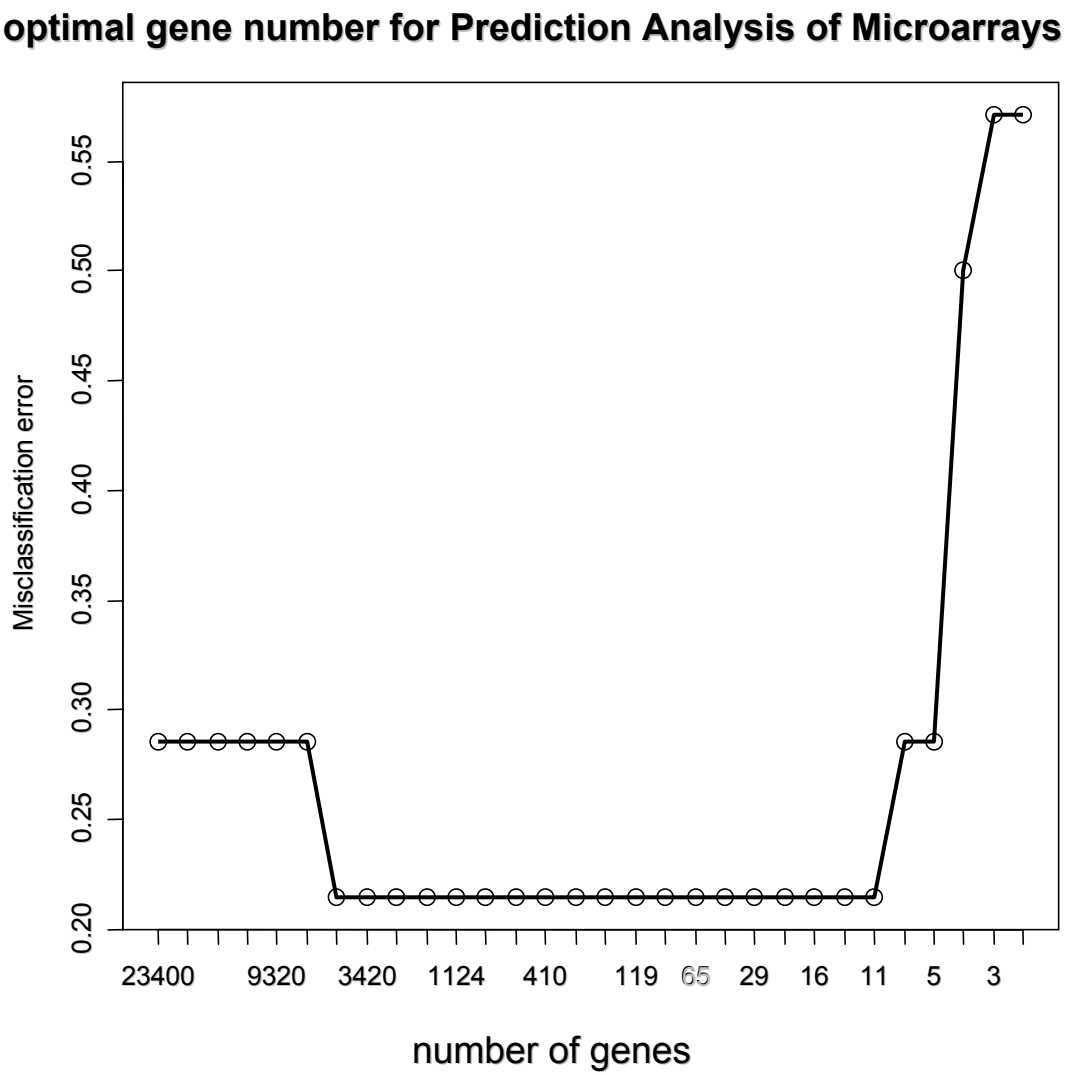
- *Tumor cell lines*
  - ***growth curves, IC50s; identified responder and non responder cell lines***
  - ***array profiles in triplicate arrays***
  - ***Genomic DNA (epigenomics, sequencing)***
  - ***Kinase activity profiling (Pamgene)***

- *Classifier tool development and evaluation*
  - ***Signatures were identified using PAM, Genetic Algorithm (GA), Random forest and Gibbs sampling***

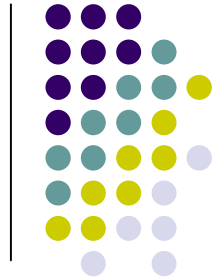
Training, Validation & Prediction



Gene Selection

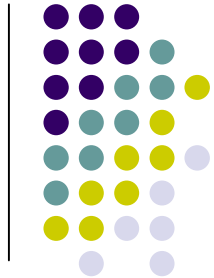


Nested-loop cross-validation



- CV :
  - Split dataset (e.g. 10 subsets) and use one as a test set
  - Train classifier on other 9 and assess predictive power
- But: which parameters to select?
  - Feature selection inside every cross-validation loop
- Result : two nested CV loops:
  - Outer one : model assessment
  - Inner one : model selection

MCRestimate Prediction



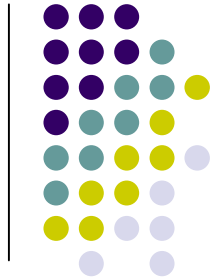
Summary of predictions for Responders

	PAM	RF	SVM
Test accuracy (%)	79	71	64
Sensitivity (%)	71	71	71
Specificity (%)	86	71	57

- Test accuracy (%): the proportion of correctly classified responders and non-responders
- Sensitivity (%): the proportion of responding cell lines identified as responders
- Specificity (%): the proportion of non-responding cell lines identified as non-responders

PAM=Prediction Analysis for Microarrays
RF=Random Forests
SVM=Support Vector Machine

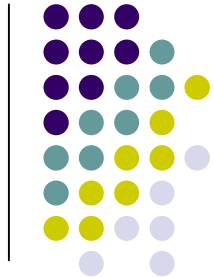
Co-primary design and analysis strategy can cope with multiple biomarkers and evolving science

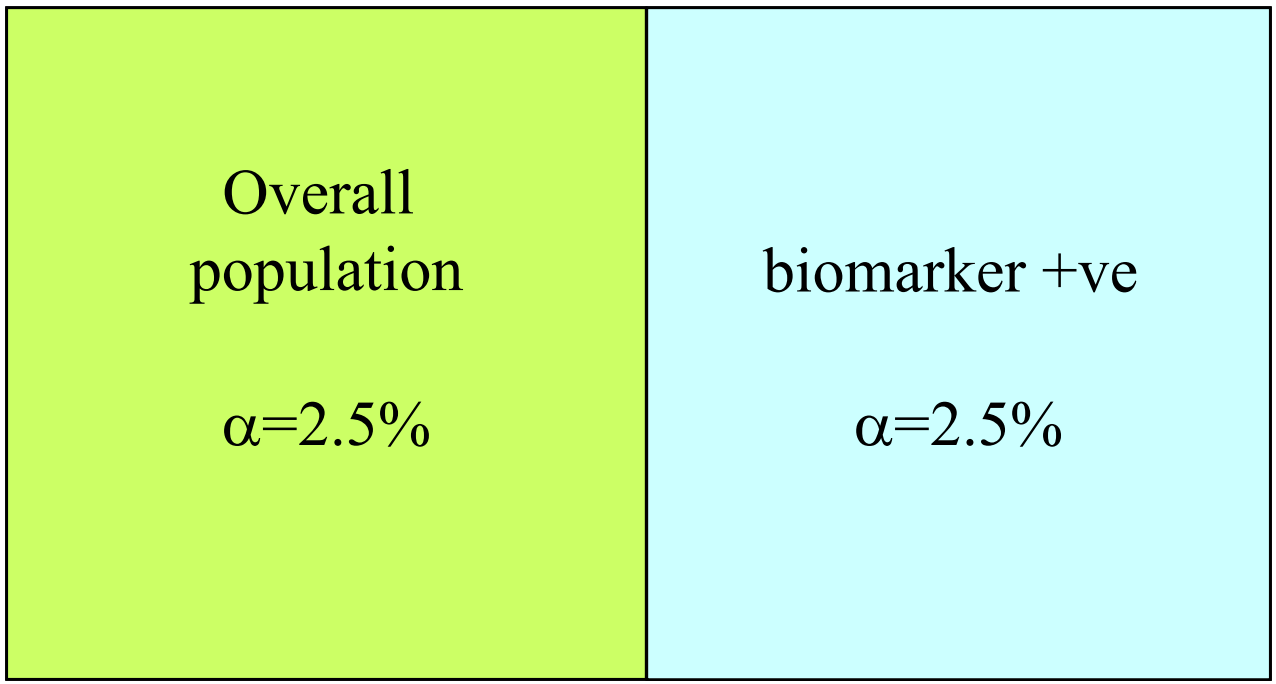


- Biomarker defined patient groups inserted as **co-primary populations** for analysis
- Analyses in co-primary populations not exploratory[1]
- P-value is shared across analyses to ensure regulatory risk is not inflated
- **Significant result in one or more of the co-primary analyses is confirmatory even if the overall trial result is not significant**
- Avoids need for a confirmatory trial and associated feasibility (and ethical) issues
- Can accommodate emerging science

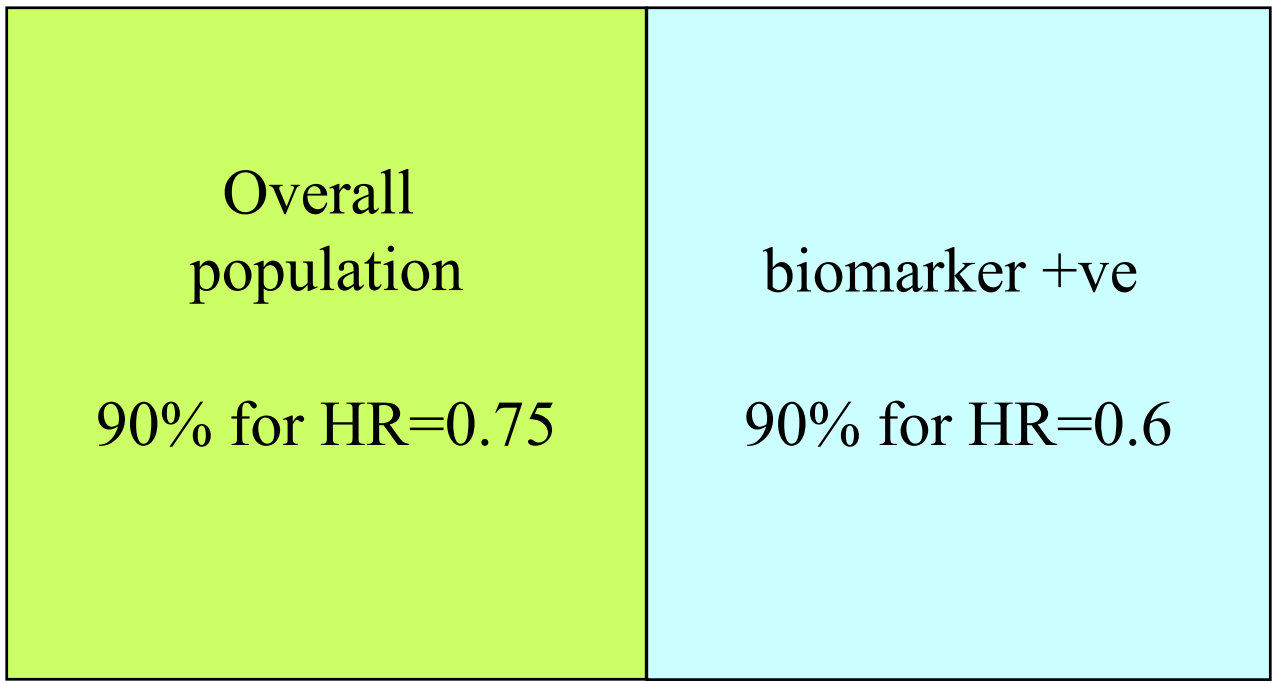
[1]Moyé and Deswal, 'Trials within Trials: Confirmatory Subgroup Analyses in Controlled Clinical Experiments' CCT 22:605–619 (2001)

Example 1: Coping with a potentially predictive biomarker

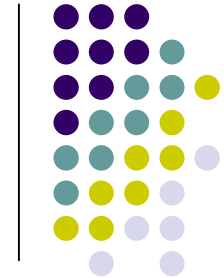


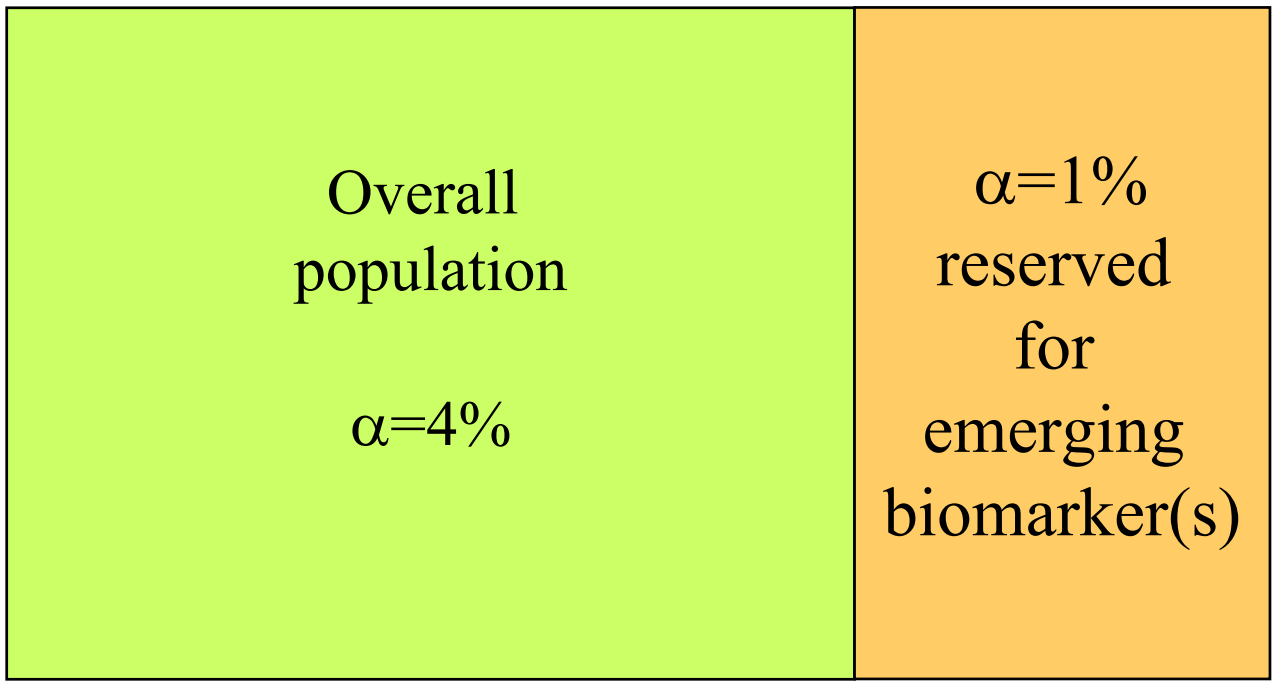


Example 1:
Power assuming one third of patients are positive for the biomarker

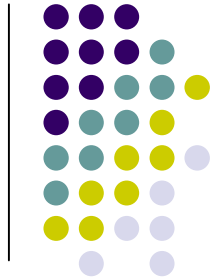


Example 2: Accommodating evolving science

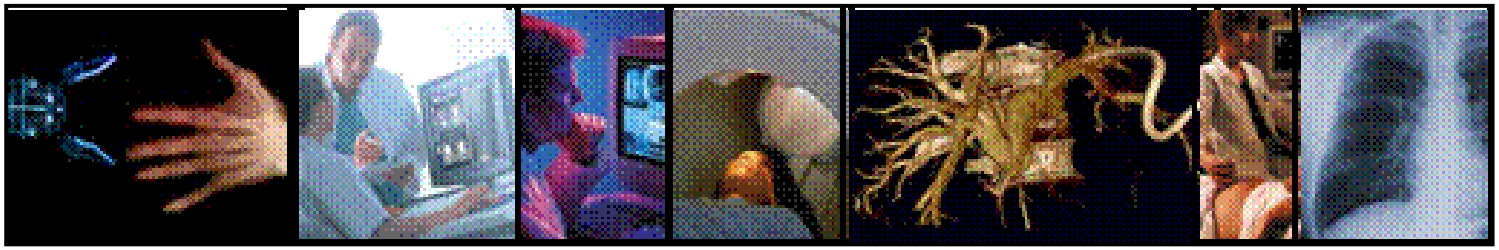




Issues



- If significance is attained in a biomarker defined co-primary population but not overall, can product labelling be considered?
- What if biomarkers are not evaluable in all patients?
- Issues will be increasingly common with targeted and pharmocgenomic drug development since heterogeneity in efficacy is likely



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