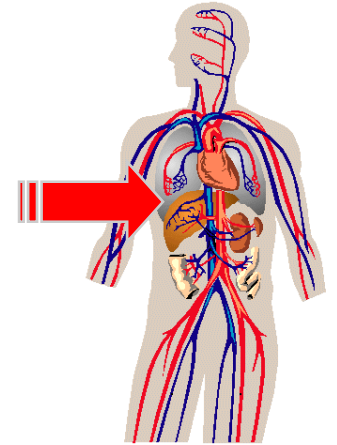


Molecular and Cellular Proteomics:
Guidelines For Clinical Proteomic Studies

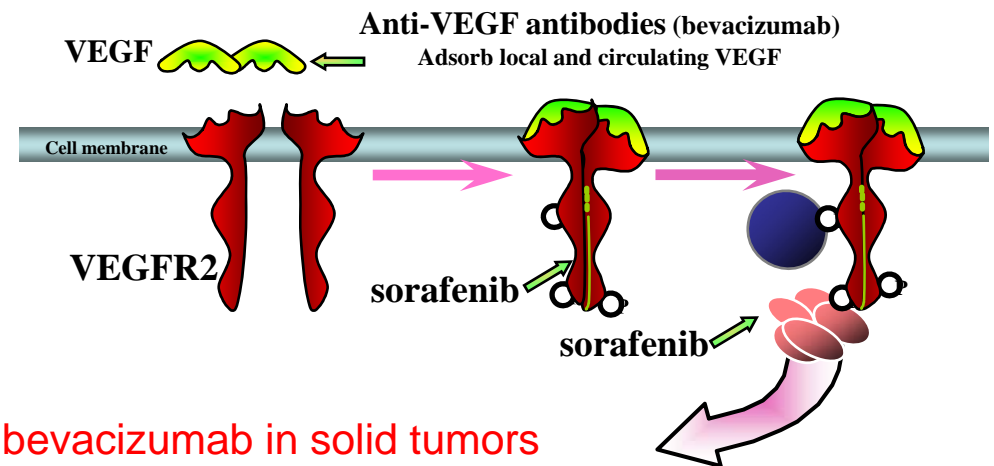


**Role of proteomics in clinical research:
Implementation of proteomics in clinical trials**

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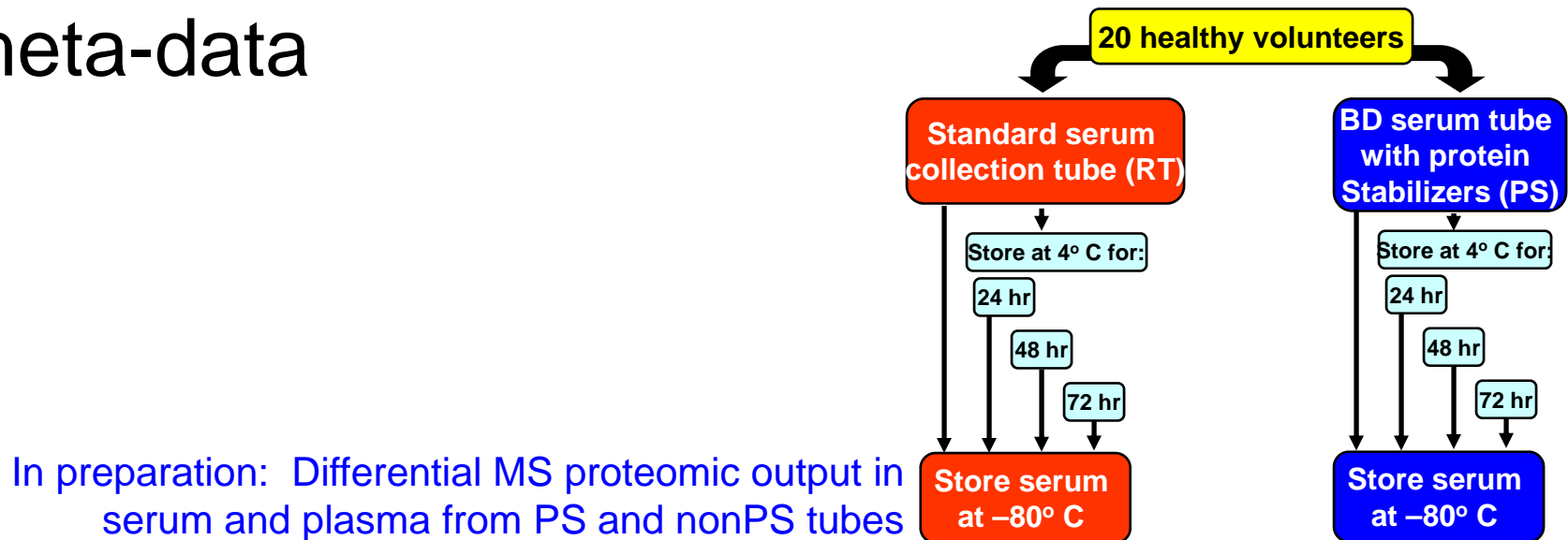
What is the role of proteomics in clinical research?

- Application: *clinical hypothesis testing*
 - Proof of concept
 - Proof of mechanism
 - Prediction
 - Prognosis
 - Diagnosis



What is the role of proteomics in clinical research?

- Implementation
 - appropriate application to hypothesis
 - consideration of design issues
 - identification and collection of data and meta-data



Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- labelling/anonymization
- sample collection and storage quality control
- validity of storage methods
- sample internal variation
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis: *does it require de novo or archival clinical material and if so what kind?*
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- sample collection and storage quality control
- validity of storage methods
- sample internal variation
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements:
 - *(re-)consent*
 - *reuse of existing samples*
 - *labelling*
 - *anonymization*
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- sample collection and storage quality control
- validity of storage methods
- sample internal variation
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size: *how was it determined? controls?*
(What is “normal”?)
- statistical power of cohort size: *considered? adequate?*
- type and number of analyses to be done: *power to address all expected analyses? subset analyses?*
- Type of sample to be ascertained
- sample collection and storage quality control
- validity of storage methods
- sample internal variation
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample: *meta-data collection*
 - *frozen v. fixed tissue*
 - *blood: plasma, v. serum*
 - *type of collection tube(s)*
 - *controls*
- sample collection and storage quality control
- validity of storage methods:
- sample internal variation:
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- sample collection and storage quality control
 - *time to and sizes of aliquots*
 - *time to processing and storage*
 - *time to and type of fixation*
 - *time to and vehicle for embedding*
- validity of storage methods:
- sample internal variation:
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- sample collection and storage quality control
- validity of storage methods:
 - *vehicle for sample storage*
 - *type of long term storage*
 - *duration of long term storage*
 - *shipping concerns*
- sample internal variation:
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- quality control for sample collection and storage validity of storage methods
- sample internal variation: *how controlled?*
 - *within and between patients*
 - *choice of patient sample*
 - *treatment or intervention*
- validation of results

Patient Samples: ISSUES

- Clinical hypothesis
- IRB/Ethics Committee requirements
- cohort size
- statistical power of cohort size
- type and number of analyses to be done
- Type of sample to be ascertained
- quality control for sample collection and storage validity of storage methods
- sample internal variation
- validation of results:
 - *unidimensional data?*
 - *how was cut-off determined?*
 - *independent training and testing/validation sets?*
 - *masked validation sets?*
 - *univariate and multivariate analyses?*
 - *biological v. technical replicates? How many is enough?*

Existing Criteria:

- **CONSORT 2001:**
 - (randomized) clinical trial checklist
 - addresses statistical power
 - patient eligibility, control, matching
 - balance of intervention
- **REMARK:** criteria for reporting biomarker studies
 - patient type, balance, demographics
 - statistical power, approaches, analyses
 - sample collection processing
 - analytical information
- **pending MIAPE 2007:** *“sufficiency and practicability”*
To include study design and sample generation, incl origin, preprocessing of biological materials, sample handling

CONSORT Statement 2001 - Checklist 
Items to include when reporting a randomized trial

<i>PAPER SECTION And topic</i>	Item	Descriptor	Reported on Page #
<i>TITLE & ABSTRACT</i>	1	<u>How participants were allocated to interventions</u> (e.g., "random allocation", "randomized", or "randomly assigned").	
<i>INTRODUCTION</i> Background	2	<u>Scientific background and explanation of rationale.</u>	
<i>METHODS</i> Participants	3	<u>Eligibility criteria for participants</u> and the <u>settings and locations where the data were collected.</u>	
Interventions	4	<u>Precise details of the interventions intended for each group and how and when they were actually administered.</u>	
Objectives	5	<u>Specific objectives and hypotheses.</u>	
Outcomes	6	<u>Clearly defined primary and secondary outcome measures</u> and, when applicable, any <u>methods used to enhance the quality of measurements</u> (e.g., multiple observations, training of assessors).	
Sample size	7	<u>How sample size was determined</u> and, when applicable, <u>explanation of any interim analyses and stopping rules.</u>	
Randomization -- Sequence generation	8	<u>Method used to generate the random allocation sequence, including details of any restrictions</u> (e.g., blocking, stratification)	

Consort Statement 2001- checklist (contd)

Randomization -- Allocation concealment	9	<u>Method used to implement the random allocation sequence</u> (e.g., numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	
Randomization -- Implementation	10	<u>Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.</u>	
Blinding (masking)	11	<u>Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment.</u> If done, <u>how the success of blinding was evaluated.</u>	
Statistical methods	12	<u>Statistical methods used to compare groups for primary outcome(s); Methods for additional analyses,</u> such as subgroup analyses and adjusted analyses.	
<i>RESULTS</i> Participant flow	13	<u>Flow of participants through each stage</u> (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. <u>Describe protocol deviations from study as planned, together with reasons.</u>	
Recruitment	14	<u>Dates defining the periods of recruitment and follow-up.</u>	
Baseline data	15	<u>Baseline demographic and clinical characteristics of each group.</u>	
Numbers	16	<u>Number of participants (denominator) in each group</u>	

REMARK Criteria:

Recommendations of the NCI and EORTC for reporting tumor marker prognostic studies

- 1. Study patient description, including source, inclusion/exclusion criteria, disease stage, and comorbidities?**
- 2. Patient treatments description, including how the treatments were chosen (randomized, rule-based)?**
- 3. Specimen description, including methods of preservation, storage, and use of control specimens?**
- 4. Assay description, including:**
 - a. detailed protocol, including specific reagents or kits*
 - b. quality control procedures*
 - c. reproducibility estimates*
 - d. quantification methods*
 - e. scoring or reporting protocols*
 - f. whether and how assays were performed and blinded to study endpoint*
- 5. Detail of selection of cases/controls, including: prospective vs. retrospective selection? stratification or matching?**

REMARK Criteria:

Recommendations of the NCI and EORTC for reporting tumor marker prognostic studies

- 6. Time period over which cases chosen identified? Span and mean of the follow-up period?**
- 7. Is there a strong rationale for sample size?**
- 8. Statistical methods clear and valid? include details of any variable selection procedure or model building procedure. How is missing data handled and how were end points determined?**
- 9. Are results study logically presented? Is the flow of patients through the study, number of patients per arm, and reasons for dropouts clearly stated? Are the distributions of demographic characteristics, standard prognostic variables, and tumor markers presented?**
- 10. Are both univariate and multivariate analyses presented?**
- 11. Are calculations of sensitivity, specificity, and predictive value included when appropriate?**

LANCET CLINICAL TRIAL CRITERIA

Aim(s)

Design (eg, randomised, parallel-group, double-blind), including:

Inclusion and exclusion criteria

Intervention(s) or method

How randomised (eg, call to central office; for RCTs)

How allocation is concealed (for RCTs)

Primary and any secondary endpoint(s)

Statistical analysis plan, including:

Sample size

Power calculations

Type of analysis (eg, ITT)

Statistics tests

Planned subgroup analyses

Ethical issues, including:

Ethics committee approval

Informed consent form and information sheet

Interim analyses and stopping rules

Start date / Finishing date / Reporting date

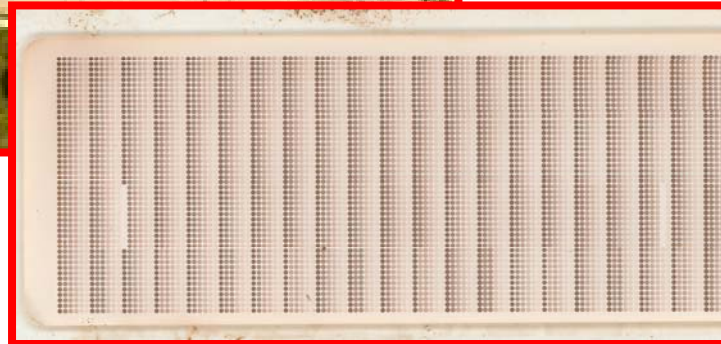
Parting Shot: Design of a prospective collection for proteomic biomarker discovery

- *Clinical hypothesis*: serum proteomics can yield a diagnostic tool to triage women with high likelihood of a malignant ovarian pelvic mass v. other pelvic mass dx
- *IRB*: received central and individual institution approval
- *cohort size*: 2000
- *statistical power*:
 - *training set*: malignant (50; P 95% for 95% sens, $\alpha=0.05$); controls (N=300; P 95% for 95% spec; $\alpha=0.05$)
 - *blinded validation set*: malignant (~100; P 93% for 95% sens; $\alpha=0.05$); controls (500; P 96% for 95% spec; $\alpha=0.01$)
- *type and number of analyses to be done*: primary is development of discriminant for ovarian cancer v. not cancer; exploratory analyses test differential of stage, grade, etc.

Parting Shot...

- *Type of sample to be ascertained:* serum
- *labelling/anonymization:* UPIN, code at statistical center
- *sample collection and storage quality control:* preop but before anesthesia, clot at RTx45 min; spin and aliquot within 4hr collection
- *validity of storage methods:* ship on dry ice then store -60 or colder at central bank
- *sample internal variation:* multiple aliquots from draw
- *validation of results:* statistical center randomly identifies cases for training v. blinded validation. Balanced for diagnosis. Statistically powered sample sizes.

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