Nonstandard Personalized Medicine Strategies for Cancer: Experimental Challenges and Alternate Interpretations

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A Different Perspective

- Mathematical biology
- Cancer therapy development
  - 23 therapies first in man
  - 5 therapies advanced to late development
    - Her3 antagonist antibody
    - Pan-alpha integrin antibody
    - Anti IL6 antibody
    - Anti IGFR antibody
    - DR5 agonist antibody
  - 2 therapies approved:
    - Topotecan for small cell lung cancer
    - Bicalutamide for adjuvant therapy of prostate cancer
- Small and large molecules targeting
  - Signal transduction
  - Repair
  - Angiogenesis
  - Developmental pathways
- DNA vaccines, immunoliposomes, antibody-drug conjugates
Objectives of the Presentation

• Briefly discuss:
  – Genetic instability of cancer
  – Non-standard personalized medicine strategies to increase the survival and cure rate of cancer patients

• Enumerate experimental challenges in translating non-standard personalized medicine

• Point out ways in which the way we do experiments may be misleading us

• Describe alternate interpretations of certain common observations
Summary

- Tumors are genetically unstable, increasing the clinical importance of evolutionary dynamics. Analysis by:
  - Efficiency of carcinogenesis
  - Predicted features of current experimental data before TCGA
- We have developed nonstandard personalized medicine strategies that significantly improve predicted survival times and cure rates
- Current personalized medicine strategies focus on:
  - average molecular properties of a tumor sample
  - at a particular point in time (usually at diagnosis)
  - with the goal of optimizing the next 1-2 therapeutic maneuvers
- Nonstandard personalized medicine strategies explicitly consider:
  - sub-clonal structure
  - evolutionary dynamics
  - risks of predicted future states
  - with the goal of optimizing the entire multi-step therapeutic plan
- Several experimental challenges must be overcome before nonstandard personalized medicine strategies can be translated
- Subtle features of our experimental systems have been influencing our theories of carcinogenesis, tumor evolution, and personalized cancer therapy
Publications


Genetic Instability of Cancer
Heterogeneity *Within* Tumors

- Tumors are genetically unstable; this is the most efficient way for cancer to evolve*
- Genetic instability leads to multiple sub-populations of tumor cells
- Resistant sub-populations are selected in response to therapy
- This is the major impediment to drug development in the age of targeted therapy

The Mutator Hypothesis

- A mutator “mutation” is any genetic/epigenetic change that leads to genetic or heritable epigenetic instability
  - Not just point mutations
- Mutator mutations leading to genetic instability play a critical role in cancer development by accelerating acquisition of cancer causing genetic/epigenetic changes
- Cells which have a mutator mutation are said to have “a mutator phenotype”
Efficiency of Cancer Evolution: A Wider Perspective*

- No one path to cancer
- All evolutionary mechanisms are themselves in Darwinian competition
- “Winners” are determined by efficiency
- Precedent in science:
  - Theory of evolution
  - Principle of Least Time (physics), Grand canonical ensemble (statistical mechanics)

Definition of Efficiency

The expected number of malignant lineages that will be generated by the mechanism by the time of life (in cell generations) at which the malignancy is typically observed.
Consequences of Efficiency

• Each mechanism is observed clinically in direct proportion to its efficiency
• Mutator mutations and selection are not mutually exclusive
  – Most efficient pathways use both
Generalizability

• When a theory is false, it is often because its underlying assumptions are false
• In biology, many critical assumptions are unknown
• It is desirable for the conclusions of a theory to be as generalizable over as many assumptions as possible
• Conclusions from experiments may also lack generalizability: they may be a special case peculiar to the experimental model or conditions
• Example:
  – Newtonian mechanics and special relativity
Selected Conclusions (Robust Across All Models)

- Mutator pathways predominate for most practical parameter values
- Magnitude of efficiency advantage ranges from thousands to billions
- Tumors which require fewer oncogenic mutations will not require a mutator mutation
- The optimal mutation rate for tumor evolution is greater than that for species evolution (independent support for mutator hypothesis)
Predictions Confirmed I

• Prediction: mutator mutations will be present in the vast majority of clinically observed cancers*

• Partial confirmation: p53 mutated in 96% of high grade serous ovarian cancers; 50% of high grade serous ovarian cancers have homologous recombination defects**

• Prediction: more such cases will be found as new DNA repair genes are discovered, introns are sequenced, and sequencing depth goes to the single cell level
Predictions Confirmed II

• Prediction: mutator mutations will not be important when less than three oncogenic mutations are required*
  

• Confirmation: retinoblastoma found to have driver mutations only in Rb; overall mutational burden dramatically lower than other tumors previously sequenced**:
  
Predictions Confirmed III

• Prediction: “Therapeutic resistance may also arise from divergent sub-clones with overlapping but non-identical sets of driver mutations.” *

• Confirmation: Divergent mutations in same pathway (amounting to convergent evolution) seen in same renal cell tumor primary from a single individual**:

• **Key feature of models**: different cells are evolving towards cancer in parallel and therefore the parallel evolution documented in 2012 was predicted in 2006
Non-Standard Personalized Medicine for Cancer

Clinical Significance of Genetic Instability

- Heterogeneity
- Moving Target
A Simple Model

- Two non cross resistant drugs or drug combos: Drug-1 and Drug-2 (i.e. RAF-MEK, PI3K)
- Four cell types:
  - Sensitive cell S, killed by both Drug-1 and Drug-2
  - Resistant cell R1, killed only by Drug-2
  - Resistant cell R2, killed only by Drug-1
  - Incurable doubly resistant cell R1-2
- Genetic and epigenetic transitions between cell types
- Cell growth and death affected by drugs in dose dependent manner
- Partial resistance
- Patient can have a mixture of cells, which evolves over time
- If a combination is given, dosage must be reduced due to toxicity
This Model Addresses Real Systems

• Anti-EGFR therapy in non-small cell lung cancer:
  – patients with sensitizing mutations develop resistance by EGFR T790M mutations
  – hsp90 inhibitors and other agents may address these populations
  – Sensitive subclone persists

• Vemurafenib therapy in b-raf mutated melanoma: other targeted agents may address some mechanisms of resistance outside the ras-raf-mek pathway

• FLT-3 ITD AML: different specific inhibitors available for different resistance mutations
Current Personalized Medicine: 28 months to incurable relapse

![Graph showing cell population changes over time with drug treatments and limits of detection.](image-url)
Nonstandard Personalized Medicine

![Graph showing cell dynamics over time with drug treatments and detection limits.](image)
In Silico “Clinical Trial”: 3 million “patients”

Examined 30 million parameter configurations encompassing:

- Different initial populations
- Different growth rates
- Different transition rates
- Different levels of sensitivity and resistance to drugs
- Parameters were chosen to encompass all reasonable possibilities based on preclinical and clinical literature and experience, providing:
  - A pan oncology sensitivity analysis
Strategies

• A **strategy** is a data-driven method for planning a sequence of therapies
  – When to treat with a combination and when to treat with sequential monotherapy
  – When to change therapies

• Like therapies, **strategies** may be individualized

• The simulation compared 6 strategies
  – Strategy 0 is the personalized medicine strategy: the patient is treated with the best drug for the observed predominant cell type and switched to the alternative drug upon tumor progression or relapse.
    • **note:** each “drug” may itself be a combination designed to kill a single subclone
  – Strategies 1, 2.1, 2.2, 3, and 4 (see backup for detail):
    • Used the evolutionary model to predict the total cell number and the likelihood of forming an incurable cell at the next 45 day timepoint
    • Gave therapy that minimized either total cell number or incurable cell likelihood
    • Differed in method of prioritizing total cell number vs incurable cells
Benefit of nonstandard personalized medicine is very general
## Differences between Current Personalized Medicine and Nonstandard Personalized Medicine

<table>
<thead>
<tr>
<th><strong>Current Personalized Medicine:</strong></th>
<th><strong>Nonstandard Personalized Medicine:</strong></th>
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<tbody>
<tr>
<td>Focus on average molecular characteristics</td>
<td>Minority subpopulations may be important</td>
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<tr>
<td>Focuses on current molecular characteristics and/or those at dx</td>
<td>Considers endgame, especially “penultimate states”</td>
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<td>Thinks primarily of current step</td>
<td>Attempts to think several steps ahead</td>
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<td>Mathematical optimization for signature development at current step</td>
<td>Piecewise, or even global, optimization across the treatment course</td>
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Nonstandard Personalized Medicine: High Level Conclusions

• The current strategy used for personalized therapy of cancer is not the only possible one
• Genetic heterogeneity and evolutionary dynamics can greatly influence the optimal strategy for personalized medicine
• The systematic study of non-standard personalized medicine strategies as a function of population substructure and evolutionary dynamics is an important area for investigation
  – The statement above is not obvious to the oncology mainstream
  – It’s not about this model or these strategies
• Benefits are potentially highly significant and very general across a large variety of tumor and therapy characteristics
Testing the Hypothesis
What Hypothesis Are We Testing

- The evolutionary simulation identifies over one million cases out of three million simulated where evolutionarily-driven therapy is significantly superior to current personalized medicine paradigms
  - An important theoretical goal is to cluster these many cases to identify a manageable number of hypotheses about cancer therapy. The simulation might contain up to one million testable hypotheses. At least, it is unlikely that each of these cases teaches the same lesson
- One testable hypothesis has clearly been identified in the example case given in the Beckman, Schemmann, Yeang PNAS 2012 paper on non-standard personalized medicine:
  - The hypothesis in this case is that a rare subclone should be the priority for treatment if it is at high risk for acquiring resistance to all available agents ("incurability"). Treatment according to the model will give the counterintuitive recommendation to first eradicate this high risk sub-clone unless the tumor burden is acutely life-threatening
Experimental Validation: Specific Aims

- Identify two or more non-cross resistant targeted agents of clinical relevance
- Develop cells that are sensitive and singly resistant to each of these agents (in vitro) (may use Schlegel methods)
  - Tag cells with lentiviral integration (DNA barcoding), fluorescent tag or other methods
  - Measure growth, death, drug sensitivity parameters for these cell types (in vitro)
  - Measure heritable transition rates between phenotypic states (may need to increase mutation rate; methods are available)
  - Molecular characterization of resistance mechanisms
- In vitro or in vivo (mice or zebrafish):
  - Establish transplant models doped with predefined ratios of sub-populations
  - Test and iteratively refine strategies using tagged cells
Challenges

• Deconvoluting molecular properties of mixtures
• Detecting and tracking minor and rare subclones:
  – One cell in 100K changed the optimal strategy!
• Determining purity of cellular reagents to this level
  – Are mutations acquired or pre-existing?
• Observing genetic evolution in short term experiments
• Evaluating dynamic properties from static data
• Predicting functional properties of drug sensitivity and growth from molecular data
• Non-invasive methods for patients
  – CTCs: need more sensitivity
  – Plasma DNA: need to know which cells have multiple resistance mutations
Estimating Genetic Diversity

- The smallest observable tumor nodules contain 1 billion cells
- Limited sequencing depth means we are observing only the tip of the diversity iceberg
- Few public databases have significant sequencing depth
- Solutions:
  - Single cell sequencing
  - Duplex sequencing*

Observing Genetic Evolution in the Lab

- Many laboratory studies are uncovering non-genetic resistance mechanisms
  - These are clearly important
  - But they occur on a more rapid time scale
- Are laboratory experiments tuned to preferentially observe short term phenomena?
- Genetic evolution is proportional to the number of **cell divisions** (**not cell generations or doublings**)
- A human tumor goes through perhaps 1000X more cell divisions before patient death than are evaluated in a mouse experiment
- To study genetic evolution in the lab, time must be “sped up” by transducing the cells with error prone DNA polymerases that enhance the mutation rate
Common and Alternative Interpretations I

• Common interpretation: genetic instability occurs late in tumor development

• Alternate interpretation: genetic instability occurs *early in a minority of cells* and is not observable in the mixture until these cells are progressively enriched (because they acquire subsequent oncogenic mutations more rapidly)
Common and Alternative Interpretations II

• Common interpretation: tumor evolution is a single branching tree from a single founder cell. The single common trunk is where therapy should be targeted.

• Alternate interpretation: parallel evolution begins early in carcinogenesis and may produce a forest rather than a single branching tree. In other instances, a different order of branching may lead to different trunks in different individuals. Parallel pathways converge on a common malignant phenotype
  – The largest tree may predominate in a mixture, but other smaller trees may become clinically significant and grow out in the face of therapy.
Common and Alternative Interpretation III

• Common interpretation: most tumor genetic diversity is pre-existing at diagnosis

• Alternate interpretation:
  – Tumor genetic diversity is proportional to the number of cell divisions, approximately proportional to the number of cells
  – At death, the number of cells may be 100-1000 fold that at diagnosis
  – Significant opportunity exists for further evolution beyond diagnosis as a result of the larger number of cell divisions which occur after dx
  – This evolution primarily generates minority subclones which have multiple resistance alleles in response to therapy. Many of these alleles were pre-existing, but not in the same cell.
  – Single cell sequencing of very large cell populations would be required to observe this. These multiply resistant cells may be more common at autopsy
Common and Alternative Interpretation IV

• Common interpretation: If we kill off the sensitive cells, the resistant cells come roaring back, because we made space for them (assumes tumor cells are competing for a finite ecological niche)

• Alternate interpretation:
  – The resistant cells either evolved (acquired resistance) or were pre-existing
  – When the resistant cells were a minority subclone, their growth was not apparent in bulk serial growth measurements
  – The growth of resistant cells is thus more obvious when the sensitive cells are removed
  – Tumor cells may not be competing for a finite ecological niche; their niche keeps expanding by metastasis until death of the host
Acknowledgements

• Non-standard personalized medicine:
  – Chen-Hsiang Yeang, Gunter Schemmann

• Genetic instability of cancer: Lawrence Loeb, Alfred Knudson

• Work initiated while CHY and RAB were members (CHY,RAB) and visitors (RAB) at the Simons Center for Systems Biology, Institute for Advanced Study (IAS), Princeton, NJ