Development and clinical application of an integrative genomic approach to personalized cancer therapy

Rong Chen, Ph.D.
Director of Clinical Genome Informatics
Icahn School of Medicine at Mount Sinai
New York, NY
http://rongchenlab.org

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Icahn School of Medicine at Mount Sinai

ChenLab
Clinical Genome Informatics

Personalized cancer therapy (PCT)

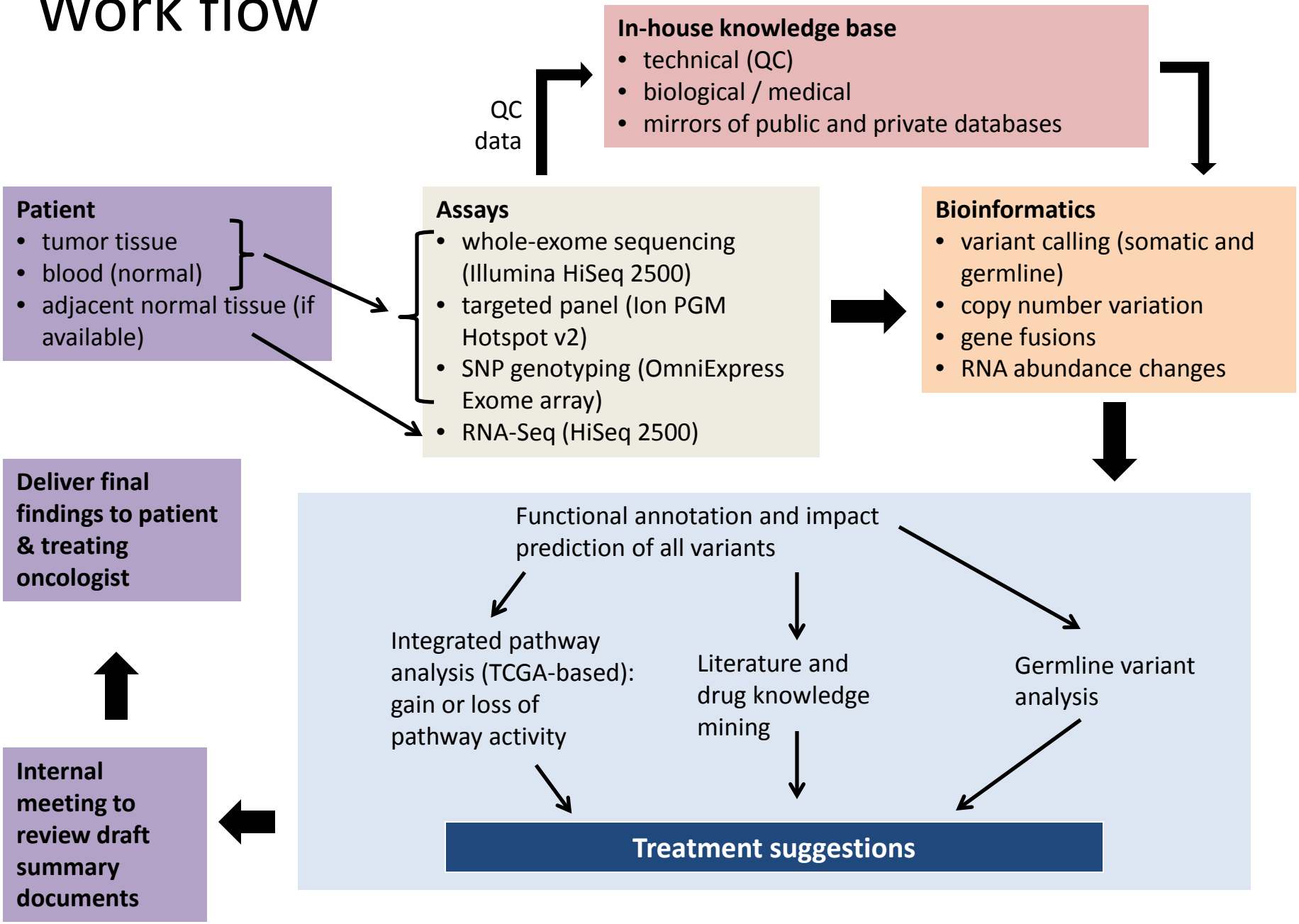
- Goal
  - recommend personalized therapeutics, clinical trials for each cancer patient based on her/his genetic and genomic profiles

- Experiments
  - Tumor: WES, genotyping, RNA-Seq
  - Blood: WES, genotyping
  - Adjacent normal: RNA-Seq when available

Our cohort of patients who received genomics reports

Characteristics	Patients (N=46)
Age at diagnosis of most recent primary (median and range, years)	48 (12-69)
Sex	
Women	26 (56.5%)
Men	20 (43.5%)
Cancer type	
Colorectal	18 (39.1%)
Other (single-primary)	7 (15.2%)
Breast	6 (13.0%)
Multiple primaries	6 (13.0%)
Medullary thyroid carcinoma	5 (10.9%)
Unknown primary	4 (8.7%)
Had metastatic disease at diagnosis	
Yes	21 (45.7%)
No	23 (50.0%)
Unknown	2 (4.3%)
Sequenced tumor specimen type	
Primary	22 (47.8%)
Metastatic	13 (28.2%)
Unknown	4 (8.7%)
Primary and metastatic	3 (6.5%)
Lymph node	2 (4.3%)
Primary and lymph node	1 (2.2%)
Local recurrence	1 (2.2%)

Work flow


In-house knowledge base
• technical (QC)
• biological / medical
• mirrors of public and private databases
QC data
Patient
• tumor tissue
• blood (normal)
• adjacent normal tissue (if available)
Assays
• whole-exome sequencing (Illumina HiSeq 2500)
• targeted panel (Ion PGM Hotspot v2)
• SNP genotyping (OmniExpress Exome array)
• RNA-Seq (HiSeq 2500)
Bioinformatics
• variant calling (somatic and germline)
• copy number variation
• gene fusions
• RNA abundance changes
Functional annotation and impact prediction of all variants
Integrated pathway analysis (TCGA-based): gain or loss of pathway activity
Literature and drug knowledge mining
Germline variant analysis
Treatment suggestions
Internal meeting to review draft summary documents
Deliver final findings to patient & treating oncologist

Selection of genomic assays

- gDNA < 1.5µg for either normal or tumor specimen
  - Only targeted panel assay was run
- gDNA 1.5-2.5µg for both normal and tumor
  - Both targeted panel and WES were run
- gDNA 2.0-2.5µg
  - WES libraries were attempted up to two times
- gDNA > 2.5µg
  - All assays (targeted panel, WES, and SNP microarray) were run

Molecular Analysis Summary : Tumor Classification

Analysis Summary : Predictive

Clinical Trial Connection

colon cancer

Protocol	Phase	Title	Target	Contact
NCT01750918	Phase I/II	BRAF/MEK/EGFR Inhibitor Combination Study in Colorectal Cancer	BRAF, MEK, EGFR	US GSK Clinical Trials Call Center 877-379-3718 GSKClinicalSupportHD@gsk.com
NCT01347866	Phase I	Clinical Study Of PI3K/mTOR Inhibitors In Combination With An Oral MEK Inhibitor Or Irinotecan In Patients With Advanced Cancer	MEK, PI3K/mTOR	Pfizer CT. gov Call Center 1-800-718-1021
NCT01927341	Phase Ib/II	Phase Ib/II Study of Efficacy and Safety of MEK162 and Panitumumab, in Adult mCRC Patients With Mutant or Wild-type RAS Tumors	MEK	Novartis Pharmaceuticals 1-888-669-6682
NCT02079740	Phase I/II	Trametinib and Navitoclax in Treating Patients With Advanced or Metastatic Solid Tumors	MEK, BCL2	Principal Investigator: Ryan Corcoran Dana-Farber Cancer Institute 617-726-8599 rbcorcoran@partners.org
NCT01351103	Phase I	A Study of Oral LGK974 in Patients With Malignancies Dependent on Wnt Ligands	PORCN (Wnt Signaling pathway)	Novartis Pharmaceuticals 1-888-669-6682

Strength of integrative approach

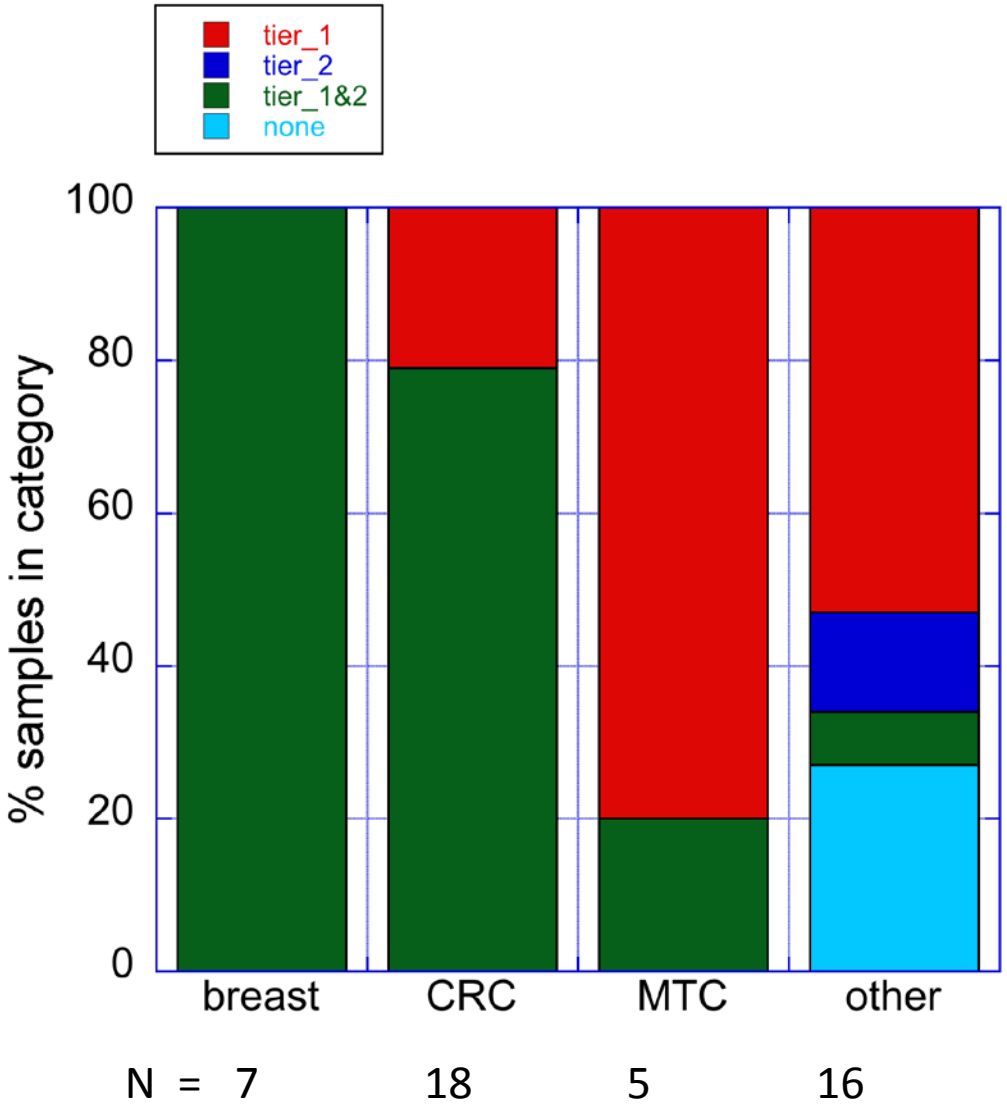
- **Identify more cancer relevant mutations and more actionable alterations.**
- Enable data interpretation at pathway level
- Identify novel or rare activating mutations
- Germline variants – pharmacogenomic biomarkers; cancer predisposing variants for prognosis and therapeutic implications.
- RNAseq – confirm SNVs/indel; prioritize/validate CNVs; cancer sub-classification; gene fusion; gene expression biomarkers without genetic level alterations.

Comparative analysis of integrative genomic approach and cancer panels

Genomic approach	Mean number of cancer-relevant somatic mutations (range)	Number of patients with tier 1 drug recommendations	Number of patients with tier 2 drug recommendations	Number of patients with actionable alterations	Mean number of actionable alterations (range)
Ion AmpliSeq Cancer Hotspot Panel v2	1.3 (0-4)	24 (52%)	16 (35%)	24 (52%)	0.65 (0-3)
Oncomine Comprehensive Panel	2.5 (0-11)	39 (85%)	24 (52%)	41 (89%)	2.4 (0-6)
FoundationOne	3.7 (0-22)	39 (85%)	24 (52%)	41 (89%)	2.6 (0-7)
This study	17.3 (1-79)	40 (87%)	26 (57%)	42 (91%)	4.9 (0-14)

Of 4.9 actionable alterations, 1.5 were somatic mutations, 0.6 were CNAs, 2.2 were germline variants, 0.7 were gene expression alterations

Actionable alterations by tumor type



Actionable = any alteration that has clinical implications for:

- **Tier 1 therapeutics**
  - FDA-approved for this cancer
- **Tier 2 therapeutics**
  - any therapeutics (including experimental) whose molecular basis of action is relevant given the patient's dysregulated pathways

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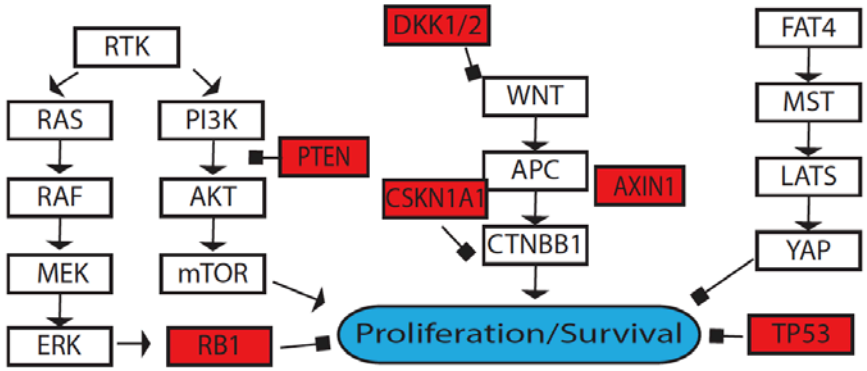
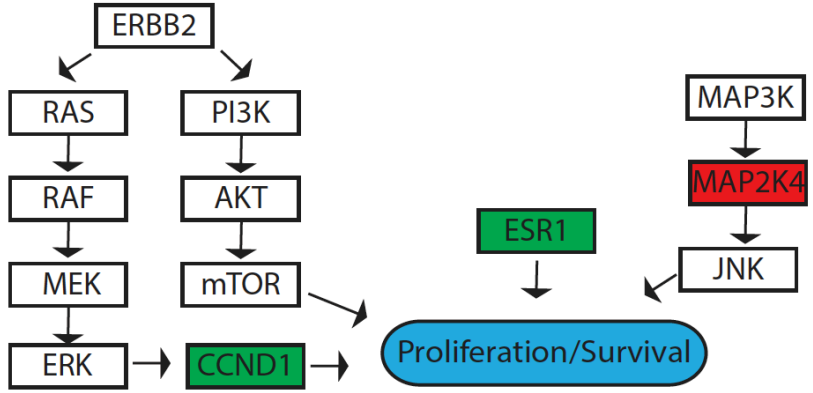
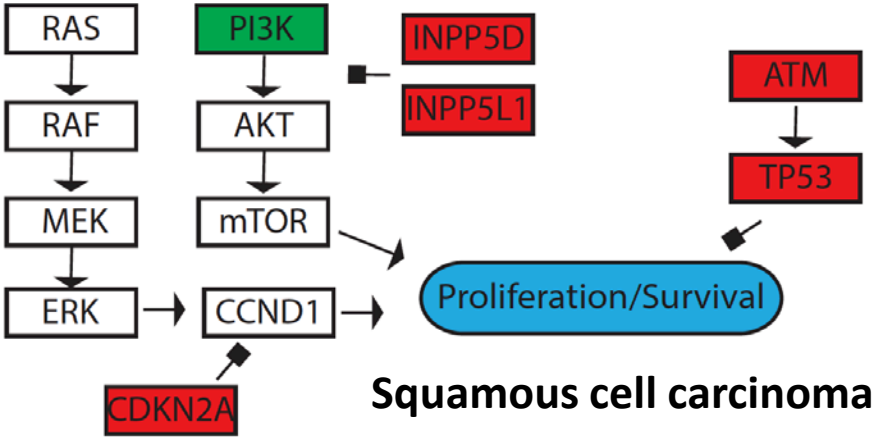
Enable data interpretation at pathway level



Colon cancer



Breast cancer (ER+/PR+/Her2-)



Squamous cell carcinoma (skin)

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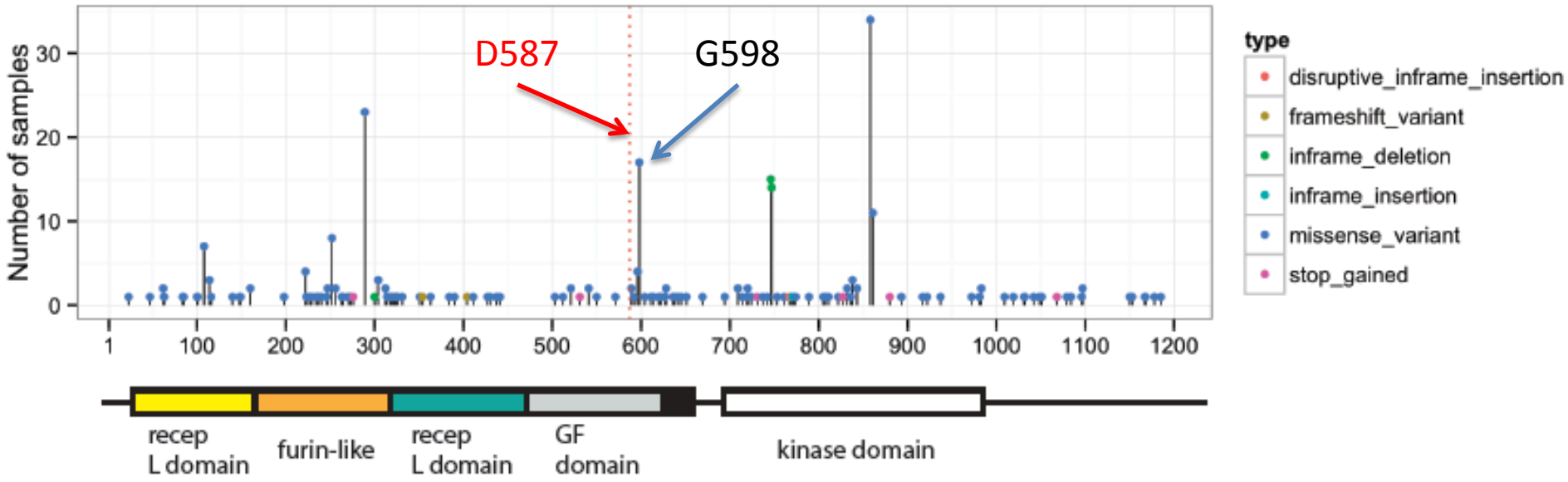
A case study

- Diagnosed with cancer of unknown primary at age 55
- Genomic analysis of a metastatic liver tumor, which was classified as poorly differentiated adenocarcinoma with signet ring features
- No known somatic mutations with available targeted therapeutic agents
- A novel EGFR D587H somatic mutation
  - Close to hotspots located at P596 and G598

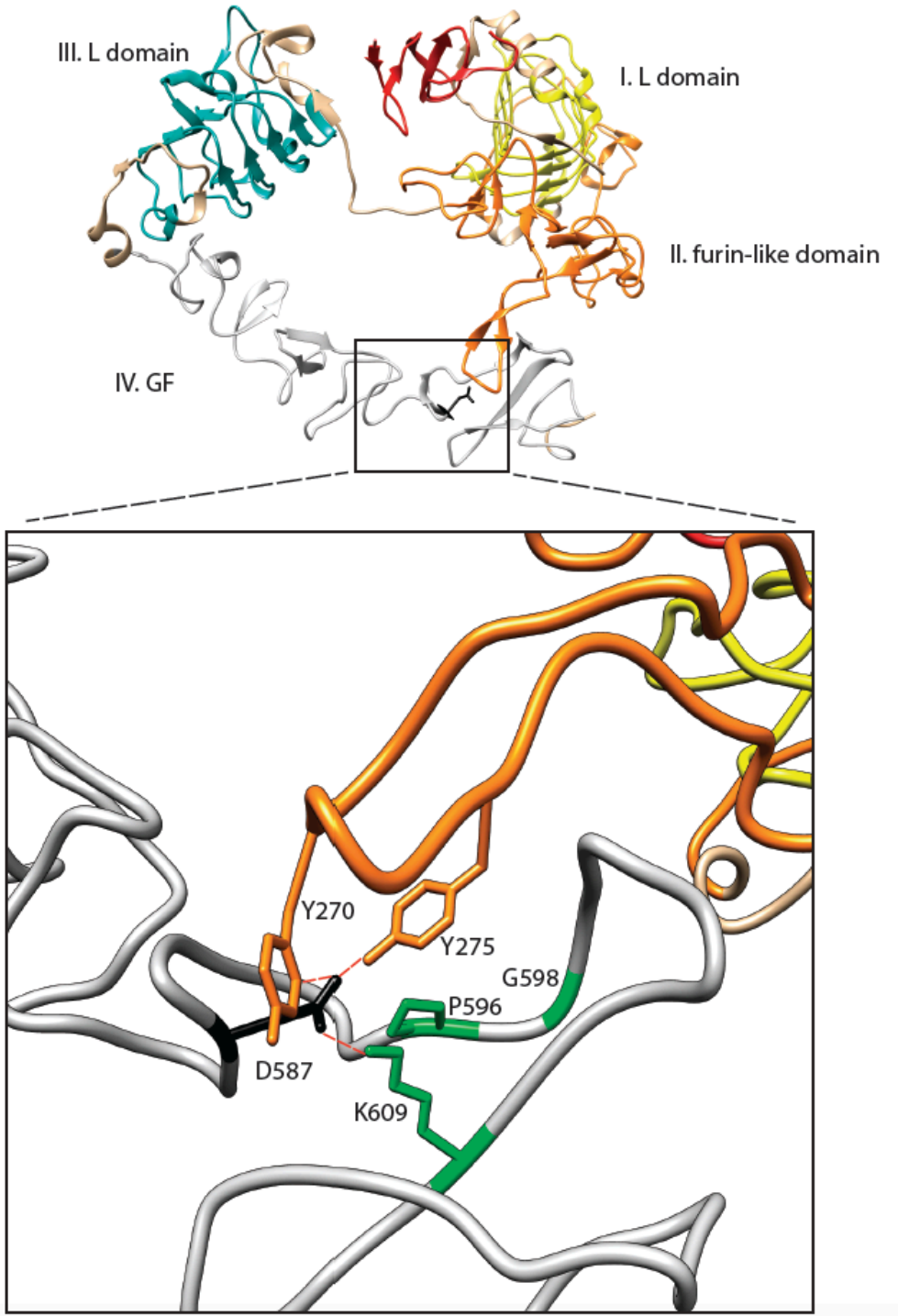
EGFR mutation frequencies from TCGA



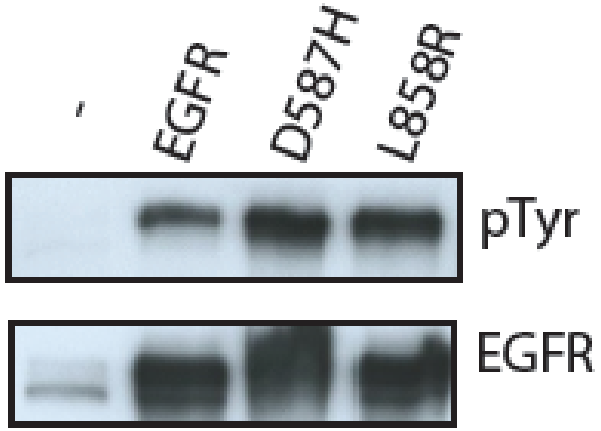
- D587 is located near hotspot at G598 within domain IV

Figure 5

B.


III. L domain
I. L domain
II. furin-like domain
IV. GF
Y270
Y275
G598
P596
D587
K609

Treatment course was changed based on a rare activating EGFR mutation



HEK293 cells

- EGFR auto-phosphorylation is augmented by D587H
- D587 activates EGFR signaling
- Recommended targeted anti-EGFR therapy
- This mutation would not be called somatic if tumor-only sequencing were performed using cancer panels

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Germline variants infers pharmacogenomics biomarkers

- A metastatic colorectal cancer case
- Genomic profiling report
  - Predicting insensitivity to cetuximab based on NRAS Q61R
  - Germline variants in KDR and CXCR2 associated with increased benefit to bevacizumab
  - Germline variants in ERCC1, ERCC2, ERCC5, XRCC1 associated with decreased benefit to oxaliplatin
- Altered treatment course
  - Treatment with bevacizumab and 5-FU resulted in brisk response that allowed for cryoablation of remaining oligometastatic lung disease
  - Initial platinum-based regimen (oxaliplatin) had limited efficacy
- Complete remission for 16 months

cancer predisposing variants for prognosis and therapeutic implications

- A breast cancer case
- Identified BRCA1 W1712fx germline variant
- Recommendation for Cisplatin chemotherapy

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- Germline variants – pharmacogenomic biomarkers; cancer predisposing variants for prognosis and therapeutic implications
- **RNAseq – confirm SNVs/indel; prioritize/validate CNVs; cancer sub-classification; gene fusion; gene expression biomarkers without genetic level alterations**

RNA-Seq augments the utility of genetic testing I

- More accurate molecular characterization
  - A breast cancer case
  - Discrepancy between pathology and RNA-Seq
    - Pathology: ER+/PR-/HER2-
    - RNA-Seq: Basal like
  - Only 10% tumor nuclei stained positive for ER, ER staining was weak (1+).

RNA-Seq augments the utility of genetic testing II

- Driver pathways are activated by abnormal expression in the absence of genetic alteration
  - A quadruple negative colon cancer case
  - Expression of EGFR ligands epiregulin and amphiregulin were elevated by 113 and 29 fold
  - Predicting favorable outcome in response to cetuximab treatment

Limitation of comprehensive integrative genomic approach

- Cost of WES and RNA-Seq are higher
- Longer time for data generation and interpretation
- Higher requirement for sample quantity and quality
- Lower sequencing depth

Recommendation

- A stagger approach
- Targeted panel sequencing first
- Progress to deeper characterization if actionable alteration are not identified
- Selecting WES depth based on initial tumor purity estimate from the panel

Follow up patient survey

- 10 patients consented for survey
  - 1 consented but chose not to respond
- 78% (7 out of 9) stated the genomic study findings met their expectation
- All 9 patients expressed some difficulty understanding the findings
- All 9 patients discussed results with their treating physicians
- 67% (6 out of 9) stated that findings are useful
- The course of treatments were altered for 4 patients

Summary

- An integrative approach to personalized cancer therapy (WES, tumor/match normal, RNA-Seq)
  - Identify more cancer relevant mutations and more actionable alterations
  - Enable data interpretation at pathway level
  - Identify novel or rare activating mutations
  - Germline variants for pharmacogenomic biomarkers, prognosis and therapeutic implications
  - RNAseq for cancer sub-classification and gene expression biomarkers without genetic level alterations
- Recommend a stagger approach

My team

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Consortium

- Uk10K
- UK Biobank
- 1000 genome
- GERA
- Wellderly
- TCGA
- ExAC
- dbGap
- …….

Thank you for your attention

Email: rong.chen@mssm.edu

Twitter: @RongChenBioinfo

Web: RongChenLab.org