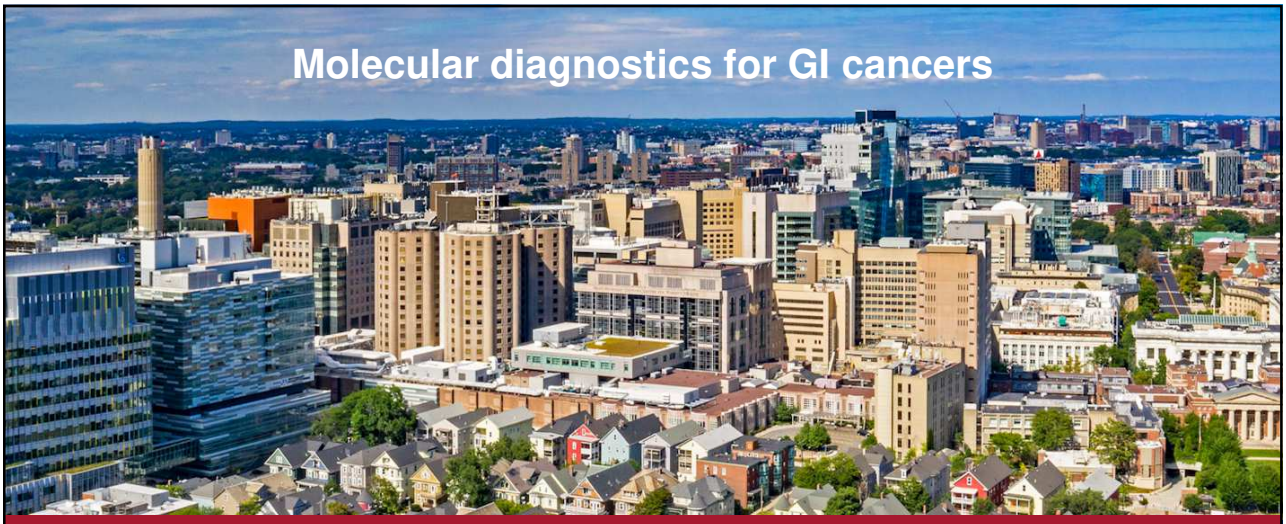
Molecular diagnostics for GI cancers



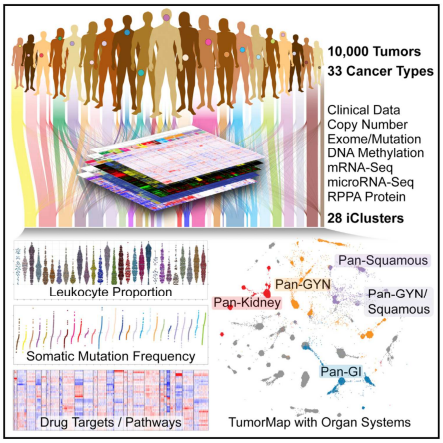
Molecular Diagnostics: Current Roles in Cancer Diagnosis and Patient Management

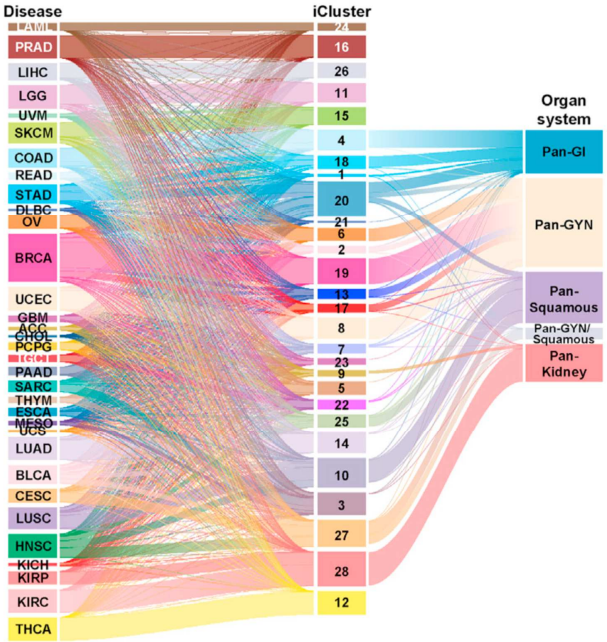
JONATHAN NOWAK, MD PhD
Associate Pathologist, Brigham and Women's Hospital
Assistant Professor, Harvard Medical School

Mass General Brigham

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

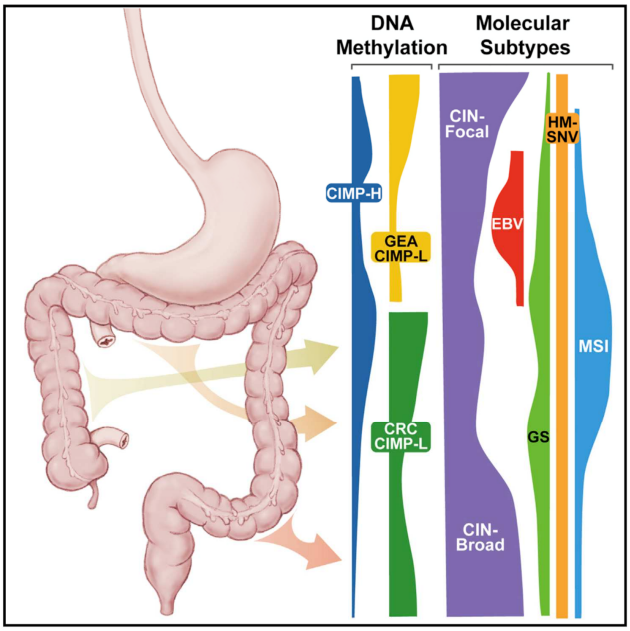
Gastrointestinal cancers share some underlying molecular features





Hoadley et al., 2018, Cell 173, 291–304. (TCGA Pan-Cancer Atlas Series)

Comparative molecular analysis of gastrointestinal adenocarcinomas



Liu et al., 2018, Cancer Cell 33, 721–735 (TCGA Pan-Cancer Atlas Series)

Outline

1. Pan-cancer molecular biomarkers
2. Gastroesophageal cancer
3. Cholangiocarcinoma
4. Pancreatic adenocarcinoma
5. Colorectal adenocarcinoma

Pan-cancer molecular biomarker approvals relevant to GI tumors

MMR Deficiency

2017

Pembrolizumab approved for unresectable or metastatic MSI-H dMMR solid tumors that have progressed following prior treatment and in patients who have no satisfactory alternative treatment options.

MSI by PCR and MMR IHC used in trial supporting approval.

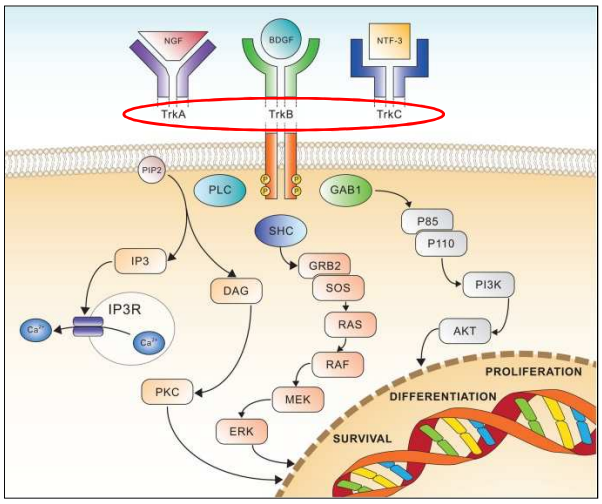
TMB-high

2020

Pembrolizumab approved for adult and pediatric patients with unresectable or metastatic tumor mutational burden-high [≥10 mut/Mb] solid tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options

FDA also approved the FoundationOne CDx assay as a companion diagnostic for pembrolizumab.

Pan-cancer molecular biomarker approvals relevant to GI tumors

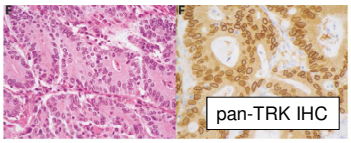


NTRK fusion

2018 and 2019

Larotrectinib and entrectinib approved for pediatric and adult patients with advanced or metastatic solid tumors with neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion without an acquired resistance mutation.

- Occur in < 1% of each major GI cancer type
- Enriched in MMR-D (MLH1/PMS2 lost), *BRAF* V600E-mutant CRC (occurs in ~5% of this subtype)
- IHC offers very good sensitivity for rearrangements; DNA / RNA NGS and FISH can also be used



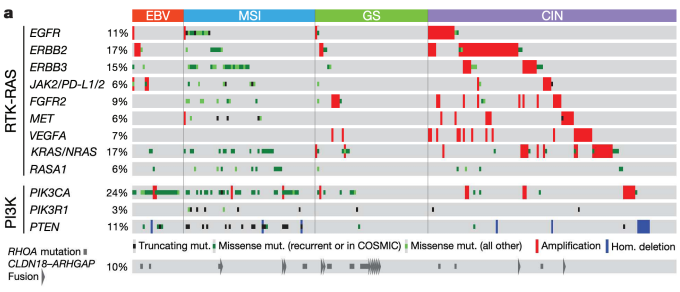
ESMO Open. 2016; 1(2): e000023.
Chou A, et al. Mod Pathol. 2019 Dec 2. doi: 10.1038

Outline

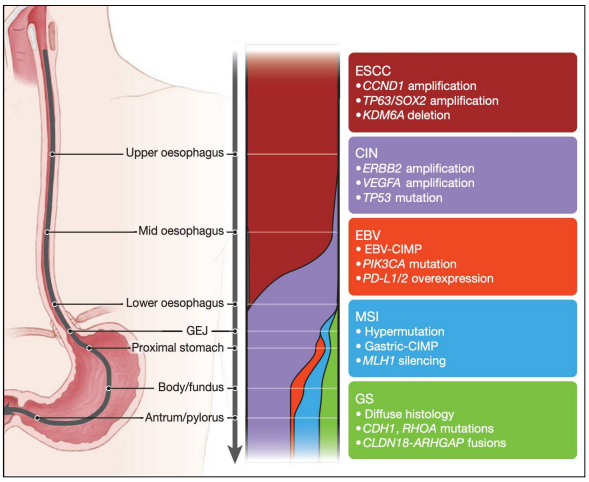
1. Pan-cancer molecular biomarkers
2. Gastroesophageal cancer
3. Cholangiocarcinoma
4. Pancreatic adenocarcinoma
5. Colorectal adenocarcinoma

Genomic landscape of gastroesophageal cancer

Integrated molecular classification of gastric cancer



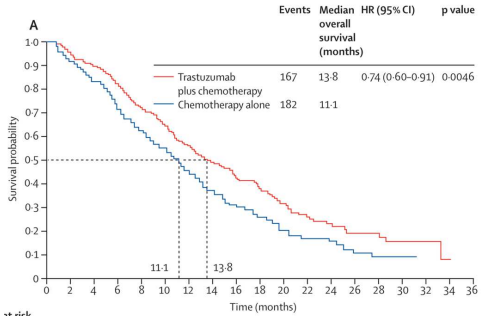
Anatomic distribution of esophageal squamous cell carcinoma and subtypes of esophageal and gastric adenocarcinoma



TCGA, 2014, Nature. 513;202-209.
TCGA, 2017, Nature. 541;169-175.

Trastuzumab for HER2+ Gastric/GEJ Cancer

ToGA Trial: Overall Survival



HER2+ Gastric/GEJ cancer

2010

Trastuzumab is indicated, in combination with cisplatin and capecitabine or 5-FU, for the treatment of patients with HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma who have not received prior treatment for metastatic disease.

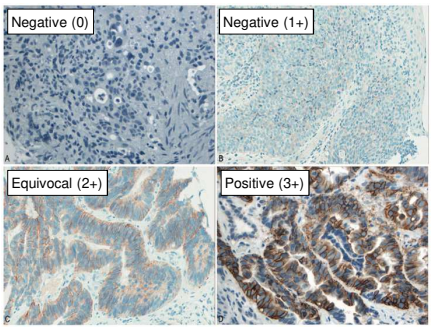
Bang YJ et al. *Lancet.* 2010;376:687-697.

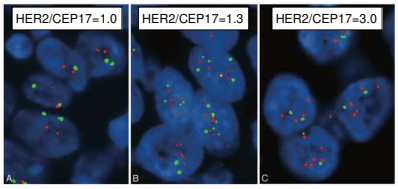
Criteria for *ERBB2* (HER2) evaluation in gastroesophageal ACA

Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 Expression Score	HER2 Expression Assessment
No reactivity or membranous reactivity in <10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	0	Negative
Faint/barely perceptible membranous reactivity in ≥10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	1+	Negative
Weak to moderate, complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a weak to moderate, complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	2+	Equivocal, reflex to FISH
Strong, complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a strong, complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	3+	Positive

Tumor cell cluster ≥ 5 neoplastic cells

<u>FISH Scoring and Assessment</u>
HER2/*CEP17* ≥2.0 positive
HER2/*CEP17* <2.0 negative

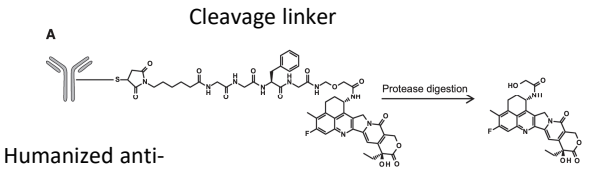




Hofmann M et al. *Histopathology.* 2008;52(7):797–805.
Bartley AN et al. *Arch Pathol Lab Med.* 2016;140(12):1345-1363.

Trastuzumab Deruxtecan for ERBB2+ Gastric Cancer

Antibody drug conjugate

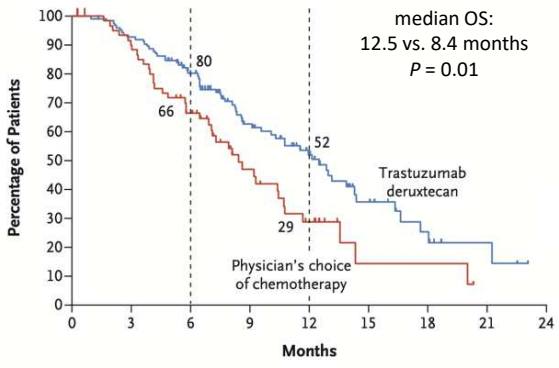


HER2+ Gastric/GEJ cancer

2021

Approved for adult patients with locally advanced or metastatic HER2-positive gastric or gastroesophageal (GEJ) ACA who have received a prior trastuzumab-based regimen.

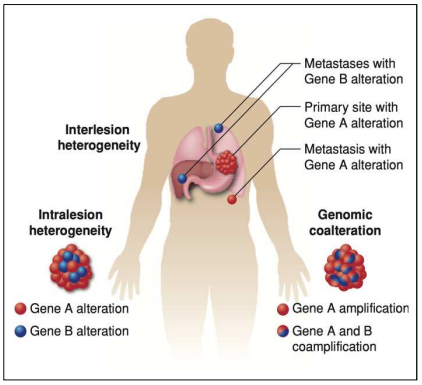
DESTINY-Gastric01 Trial

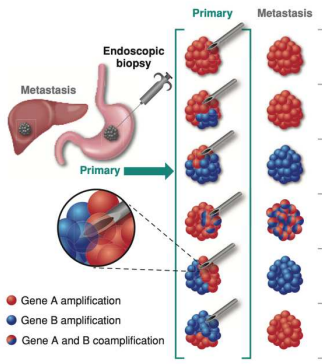


Overall survival

Clin Cancer Res. 2016 Oct 15;22(20):5097-5108.
Shitara K et al. *N Engl J Med.* 2020;382(25):2419-2430.

Notable genomic heterogeneity in gastroesophageal adenocarcinoma





Gene A FISH	Next-generation sequencing	Circulating tumor DNA	Response to Gene A–directed therapy
+	Gene A amplification	Gene A amplification	Sensitive
+	Gene A amplification	Gene A ± B amplification	Initial sensitivity but with shorter time to progression
+	Gene A amplification	Gene B ± A amplification	Resistant
+	Gene A and B amplification	Gene A and B amplification	Resistant
+ in a subset of cells	Gene A and B amplification	Gene B ± A amplification	Resistant
–	Gene B amplification	Gene A ± B amplification	Initial sensitivity but with shorter time to progression

Nagaraja AK et al. *Cancer Discov.* 2019;9(12):1656-1672.

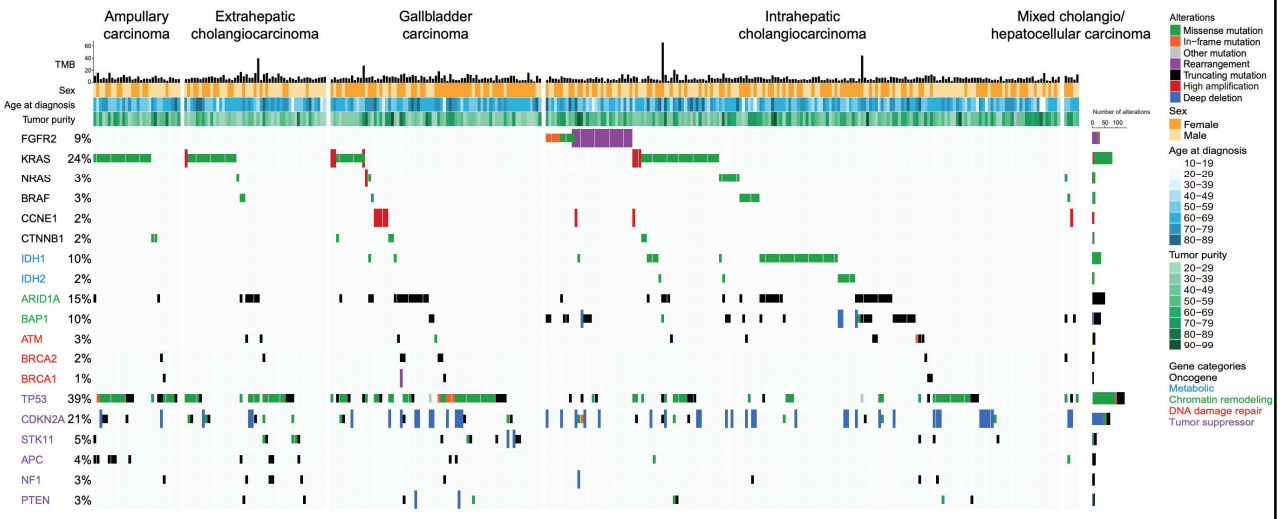
Gastroesophageal cancer summary

- Five main subtypes of cancer
- Distributed in distinct proximal-distal gradients
- Variety of driver alterations
- Evidence for substantial spatial heterogeneity in molecular alterations
- *ERBB2* amplification currently targetable
- PD-L1^{+} gastric and GEJ adenocarcinomas, as well as esophageal squamous cell carcinomas, that are recurrent and non-responsive to chemotherapy can be treated with pembrolizumab

Outline

1. Pan-cancer molecular biomarkers
2. Gastroesophageal cancer
3. Cholangiocarcinoma
4. Pancreatic adenocarcinoma
5. Colorectal adenocarcinoma

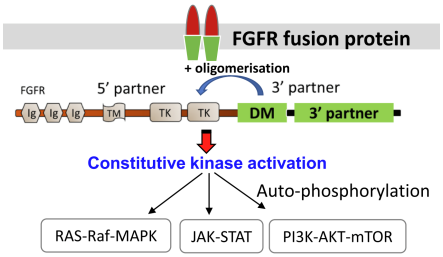
Cholangiocarcinoma landscape



Cleary JM et al. Cancer Discov. 2021 Apr 29;candisc.1669.2020.

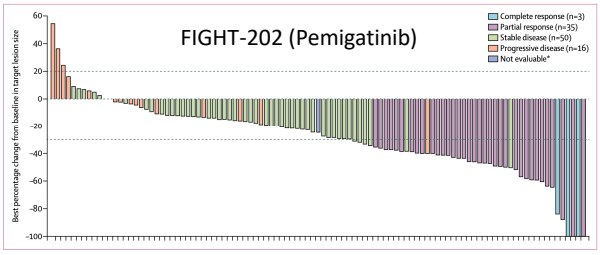
Targeting *FGFR2*-rearranged cholangiocarcinoma

Dysregulated FGFR2 signaling



- FGFR2 rearrangements present in 10-15% of intrahepatic cholangiocarcinomas
- 40+ fusion partners
- Detect via FISH, RNA sequencing, DNA sequencing

Goyal L et al. Cancer Treat Rev. 2021 Apr;95:102170.
Abou-Alfa GK et al. Lancet Oncol. 2020 May;21(5):671-684.



FGFR2-rearranged cholangiocarcinoma

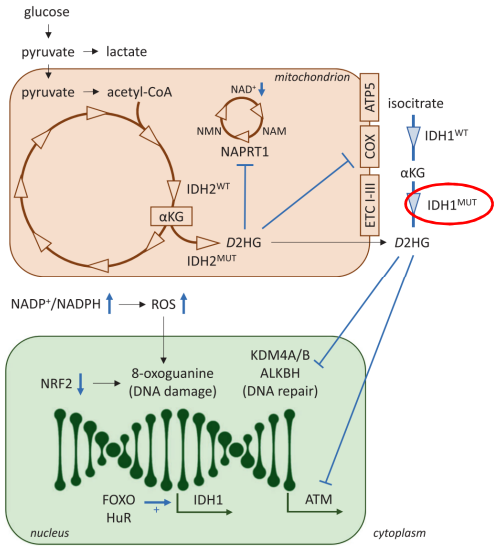
2020

Pemigatinib approved for adults with locally advanced, *FGFR2*-rearranged cholangiocarcinoma

2021

Futibatinib given breakthrough therapy status for adults with locally advanced or metastatic, *FGFR2*-rearranged cholangiocarcinoma

Targeting *IDH1*-mutant cholangiocarcinoma



- IDH1 switch-of-function hotspot mutations in ~15% of cholangiocarcinomas
- Can target via small molecular inhibitors of R132 mutant IDH1 enzyme
- ClarIDHy trial updated analysis: Median OS (mOS) was 10.3 months for ivosidenib and 7.5 months for placebo (one-sided p = 0.093).
- OS HR = 0.49 (*P* < .0001) after adjustment for cross-over

IDH1-mutant cholangiocarcinoma

2021

Ivosidenib under priority review by FDA
Decision expected late Fall 2021

Molenaar RJ et al. Oncogene (2018) 37:1949–1960
Zhu AX et al. J Clin Oncol 39, 2021 (suppl 3; abstr 266).

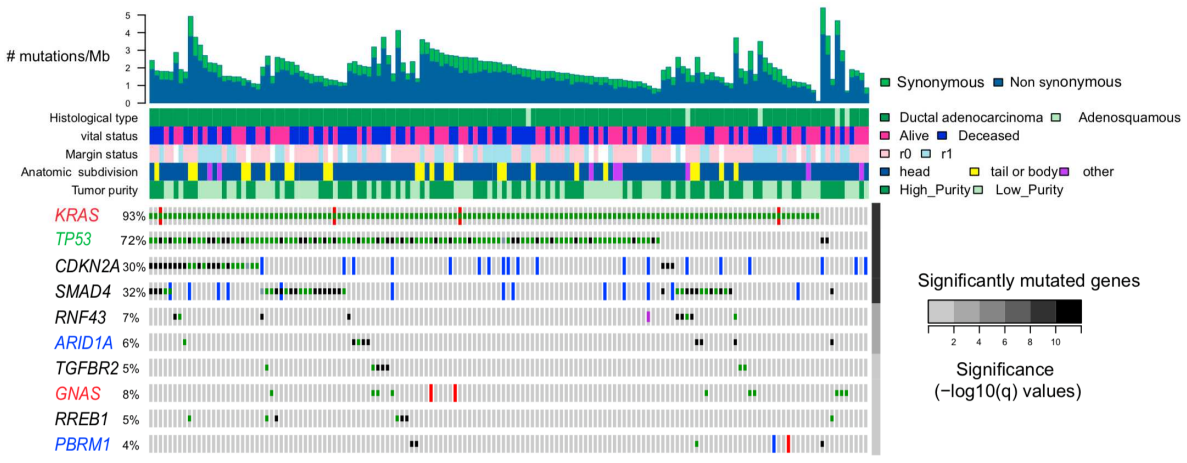
Cholangiocarcinoma summary

- Intrahepatic and extrahepatic cholangiocarcinoma are molecularly distinct
- *FGFR2* rearrangements and *IDH1* switch-of-function mutations are enriched in intrahepatic cholangiocarcinoma
- *FGFR2* rearrangements targetable with pemigatinib and futabitinib
- *IDH1* mutations shown to be targetable with ivosidenib in clinical trial; FDA review underway

Outline

1. Pan-cancer molecular biomarkers
2. Gastroesophageal cancer
3. Cholangiocarcinoma
4. Pancreatic adenocarcinoma
5. Colorectal adenocarcinoma

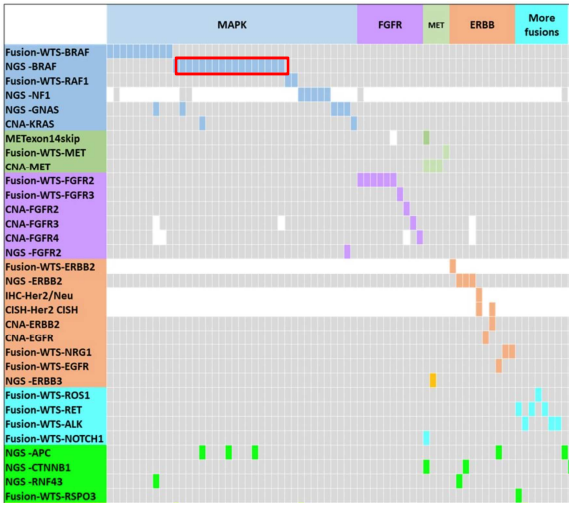
Pancreatic adenocarcinoma landscape



TCGA. 2017, Cancer Cell 32, 185–203.

Wide variety of potentially targetable alterations in *KRAS* WT PDAC

144 KRAS WT tumors → 78% with potentially targetable alterations

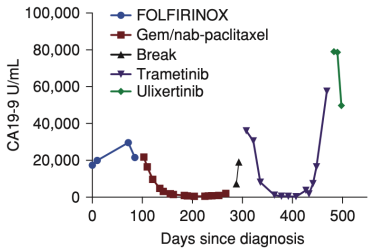


Activating *BRAF* in-frame deletions in 1% of PDAC

Involved Amino Acids: p.N486-Q494

Optimal deletion length appears to be 5 AA

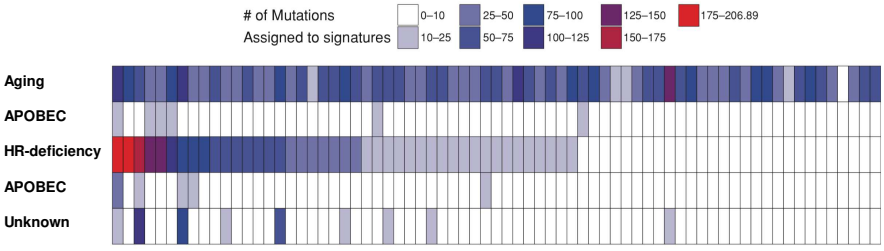
BRAF p.N486_P490del PDAC



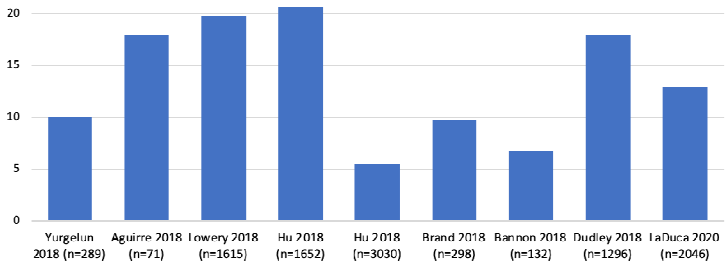
Philip PA, et al. J Clin Oncol 38: 2020 (suppl; abstr 4629). 2020 ASCO Virtual
Dankner M, et al. Oncogene (2018) 37:3183–3199. Aguirre AJ, et al. Cancer Discov. 2018;8(9):1096-1111.

Homologous recombination deficient and germline mutations in PDAC

HR deficiency signature in metastatic PDAC



Rate of germline path/likely path alterations in PDAC patients



Germline BRCA1/2 mutant PDAC

2019

Olaparib approved for germline BRCA1/2 mutant metastatic PDAC that has not progressed on first-line platinum-based chemotherapy (POLO trial)

NCCN recommends germline testing for all pancreatic cancer patients.

Aguirre AJ, et al. Cancer Discov. 2018;8(9):1096-1111. Golan T, et al. N Engl J Med. 2019;381:317-27.

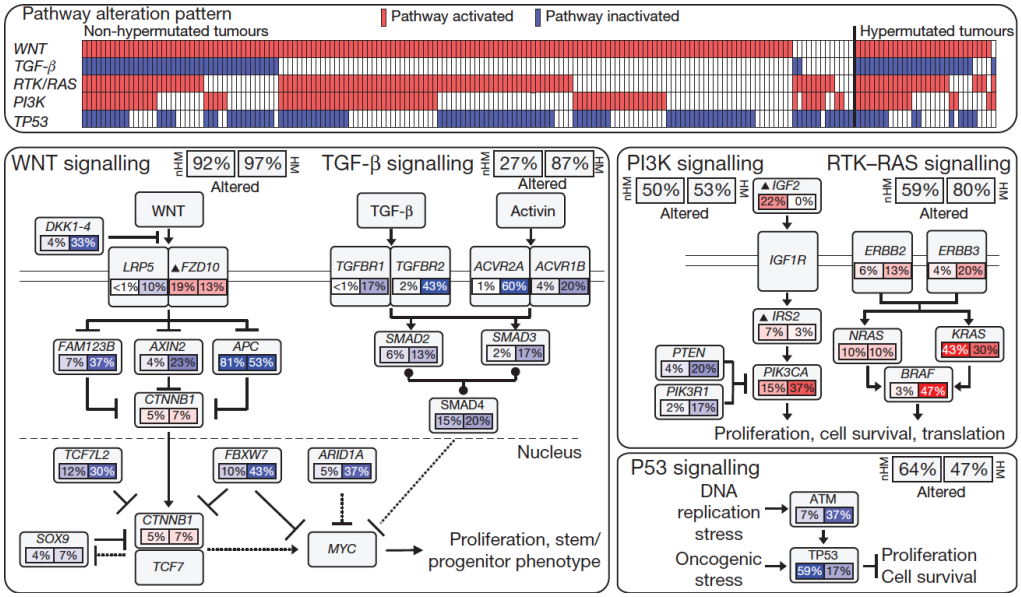
Pancreatic adenocarcinoma summary

- *KRAS* is key oncogenic driver
- Relative genomic homogeneity
- *KRAS* WT tumors have frequent, potentially targetable alterations
- 10-15% of patients have pathogenic germline alterations
- Germline *BRCA1*/*BRCA2*-mutant patients eligible for maintenance PARP inhibition with olaparib

Outline

1. Pan-cancer molecular biomarkers
2. Gastroesophageal cancer
3. Cholangiocarcinoma
4. Pancreatic adenocarcinoma
5. Colorectal adenocarcinoma

CRC Landscape



Cancer Genome Atlas Network. Nature. 2012 Jul;487(7407):330-7.

CRC and MMR Deficiency

- Chromosomal Instability Pathway
- Hypermutability Pathway

MMR-D CRC

2017/2018

Nivolumab/Nivolumab+ipilimumab approved for MSI-H / MMR-D CRC following progression on chemotherapy (CHECKMATE 142)

2020

Pembrolizumab approved as *first-line* treatment for unresectable or metastatic MSI-H / MMR-D CRC (KEYNOTE-177)

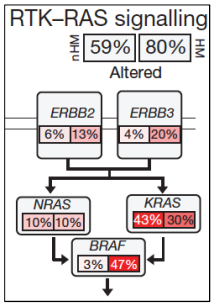
- CRCs with defective DNA mismatch repair/hypermutability arise in two major settings:
  - Sporadic (12-15%)
  - Inherited predisposition; Lynch Syndrome (3-4%)
- All CRCs need MMR testing
- Determination of the cause for MMR deficiency in CRC is necessary in order to rule in/out Lynch syndrome
  - Often requires multiple testing methodologies
- Most sporadic MMR-deficient CRC is due to *MLH1* promoter methylation; *MLH1* promoter methylation extremely rare in Lynch syndrome
- Approximately half of sporadic MMR-deficient CRCs have a *BRAF* V600E mutation; *BRAF* V600E almost never occurs in MMR-deficient tumors in Lynch syndrome patients

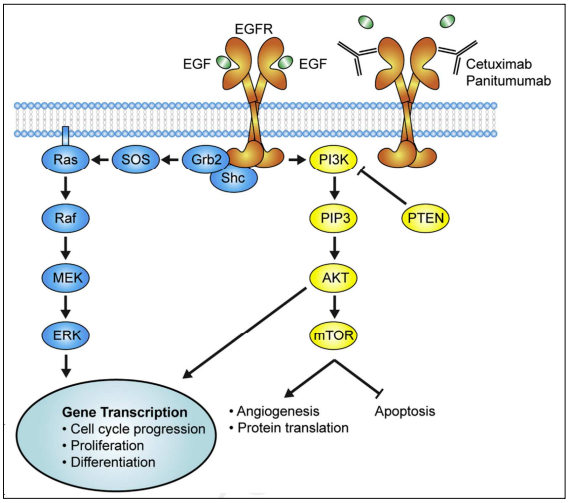
EGFR/MAPK targeting in CRC

(Returning to the dawn of solid tumor targeted therapy…)

KRAS/NRAS/BRAF WT CRC

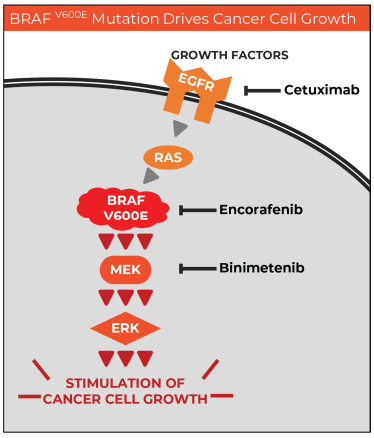
- **2004 and 2006**
  - Cetuximab and panitumumab approved for EGFR-expressing metastatic CRC
- **2007**
  - CRYSTAL: Very subtle benefit of cetuximab in EGFR-expressing metastatic colorectal cancer
- **2008**
  - CAIRO2: *KRAS* codon 12+13 mutations confer resistance
- **2012**
  - FDA modifies cetuximab approval to encompass *KRAS* wild-type, EGFR-expressing metastatic colorectal cancer
- **2013**
  - COIN: Additional mutations in *KRAS* and *NRAS* are also associated with non-response



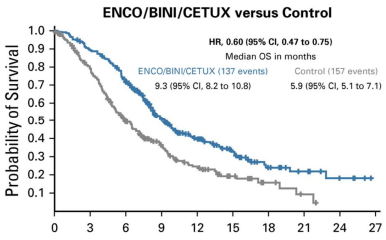


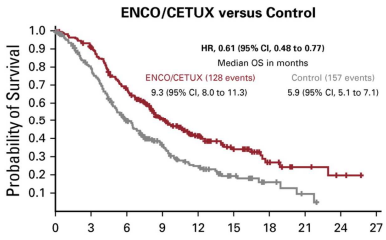
Chong, CR, et al. The quest to overcome resistance to EGFR-targeted therapies in cancer. Nat Med. 19:1389-1400. 2013.
Nowak JA and Hornick JL. Surg Path Clin. 2016;9(3):427-39.

Dual agent targeted therapy for *BRAF* V600E mutant CRC



BEACON study





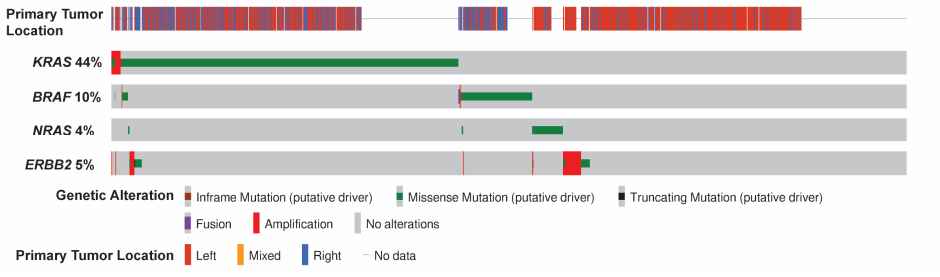
BRAF V600E mutant CRC

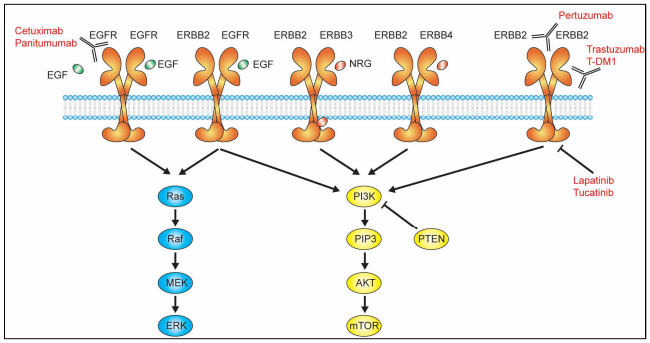
2020

Encorafenib in combination with cetuximab approved for the treatment of adult patients with metastatic BRAF V600E mutant CRC after prior therapy

J Clin Oncol. 2021. 39(4);273-284.
https://www.cancer.gov/news-events/cancer-currents-blog/2019/colorectal-cancer-braf-triplet-targeted-therapy

ERBB2 blockade for ERBB2-amplified CRC





Nowak JA. Surg Path Clin. 2020;13(3):485-502.

ERBB2 blockade for ERBB2-amplified CRC

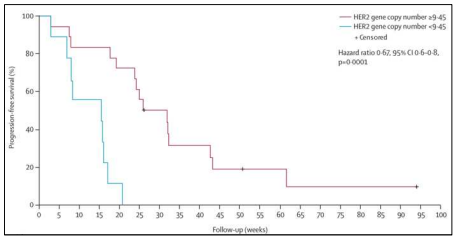
- **HERACLES A**: Phase II trial of dual HER2 blockade with trastuzumab and lapatinib for *KRAS* codon 12-13 WT, *HER2*-amplified tumors that were refractory to standard of care → 30% ORR
- **MyPathway**: Phase II trial of dual HER2 blockade with trastuzumab and pertuzumab for *HER2*-amplified tumors → 32% ORR

ERBB2-amplified, _RAS_-WT CRC

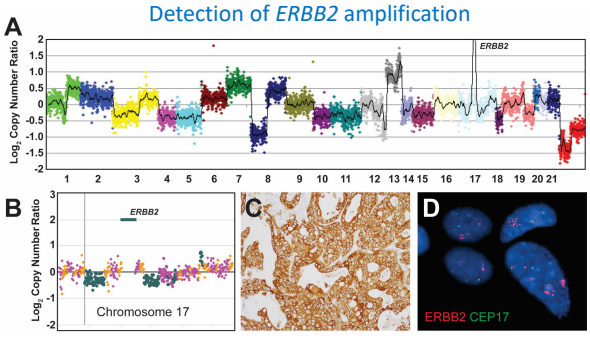
2020

NCCN guidelines recommend treatment with trastuzumab and either pertuzumab or lapatinib for *RAS* wild-type, *HER2*-amplified metastatic CRC

HERACLES A PFS by *HER2* copy number status



Detection of *ERBB2* amplification



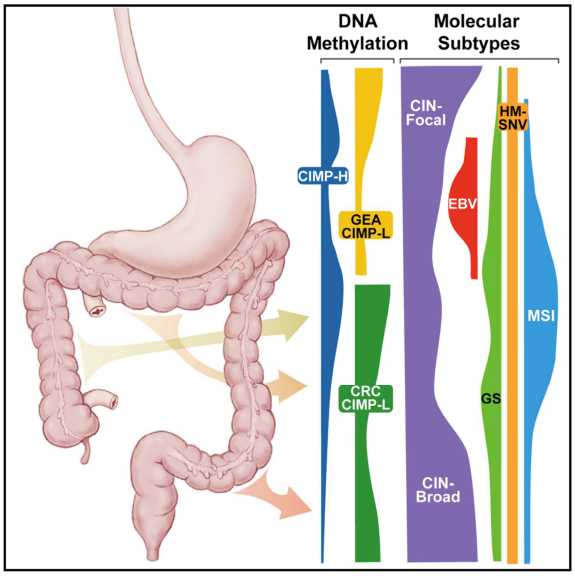
Sartore-Bianchi A et al. *Lancet Oncol.* 2016;17(6):738-746;
Meric-Bernstam F et al. *Lancet Oncol.* 2019;20(4):518-530;
NCCN. Colon cancer v 4.2020. Accessed September 5, 2020.
Nowak JA. Surg Path Clin. 2020;13(3):485-502.

CRC Summary

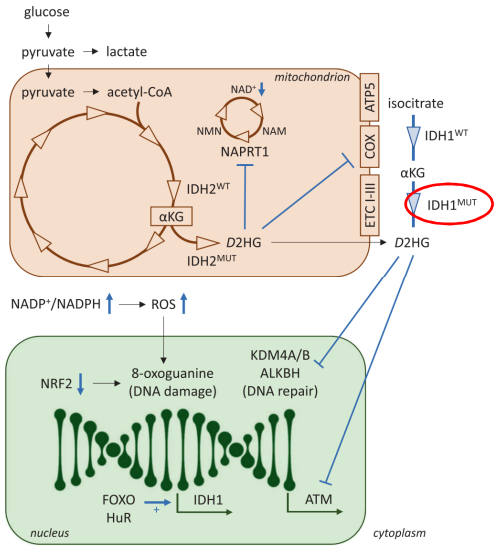
- Two major subtypes: chromosomal instability ("CIN") pathway and MMR-deficient
- Numerous RASK/MAPK driver alterations
- Universal MMR testing for detection of Lynch syndrome, prognostication and prediction of therapeutic response
  - Pembrolizumab approved in first line for MMR-deficient metastatic CRC
- Need to identify cause of MMR deficiency, if present
- Firmly established roles for *KRAS*/*NRAS*/*BRAF* analysis
  - Anti-EGFR therapy in *KRAS*/*NRAS*/*BRAF* wild-type tumors
  - Dual targeted inhibition for *BRAF* V600E mutant tumors
- Recently established role for *ERBB2* amplification

Molecular diagnostics for GI cancers

1. Pan-cancer molecular biomarkers
2. Gastroesophageal cancer
3. Cholangiocarcinoma
4. Pancreatic adenocarcinoma
5. Colorectal adenocarcinoma



September 2021 Addendum: Targeting *IDH1*-mutant cholangiocarcinoma



- IDH1 switch-of-function hotspot mutations in ~15% of cholangiocarcinomas
- Can target via small molecular inhibitors of R132 mutant IDH1 enzyme
- ClarIDHy trial updated analysis: Median OS (mOS) was 10.3 months for ivosidenib and 7.5 months for placebo (one-sided p = 0.093).
- OS HR = 0.49 (*P* < .0001) after adjustment for cross-over

IDH1-mutant cholangiocarcinoma

August 2021

Approved for adult patients with previously treated, locally advanced or metastatic cholangiocarcinoma with an *IDH1* R132 mutation

Molenaar RJ et al. Oncogene (2018) 37:1949–1960
Zhu AX et al. J Clin Oncol 39, 2021 (suppl 3; abstr 266).