PRECIZNÍ ONKOLOGIE U CRC

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MOLEKULÁRNÍ TESTOVÁNÍ

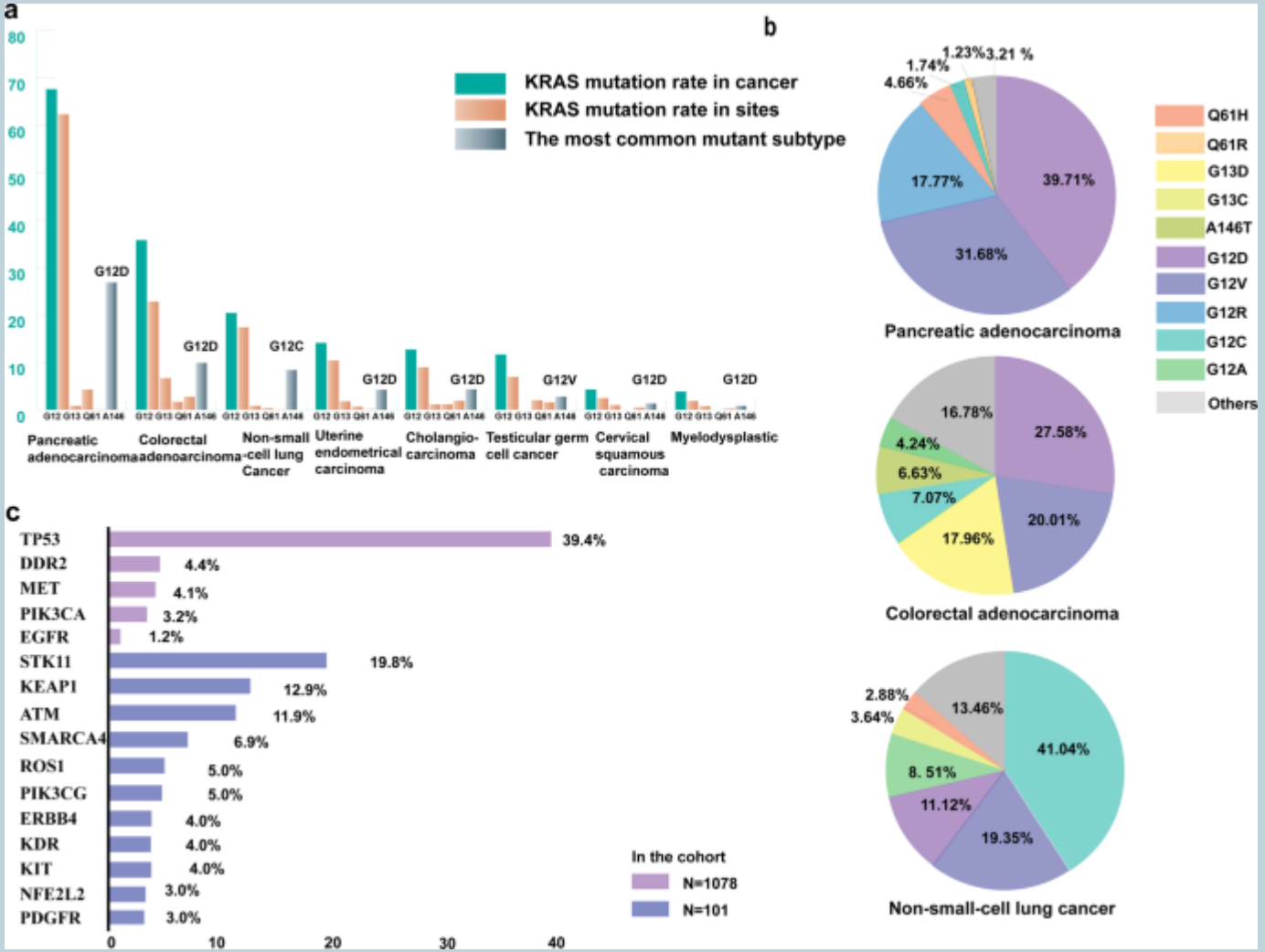
- Jak (co) testovat?
  - IHC (HER, BRAF)
  - qPCR, NGS DNA + RNA; jaký panel?
  - metylace
- Koho testovat?
  - arbitrárně všechny pacienty
  - mladé pacienty
  - vzácné diagnosy
- Kdy testovat?
  - před zahájením léčby
  - při progresi
  - při vyčerpaných možnostech

CRC STANDARD

- KRAS
- NRAS
- BRAF
- MMR
- MSI
- DPYD (UGT)

KRAS

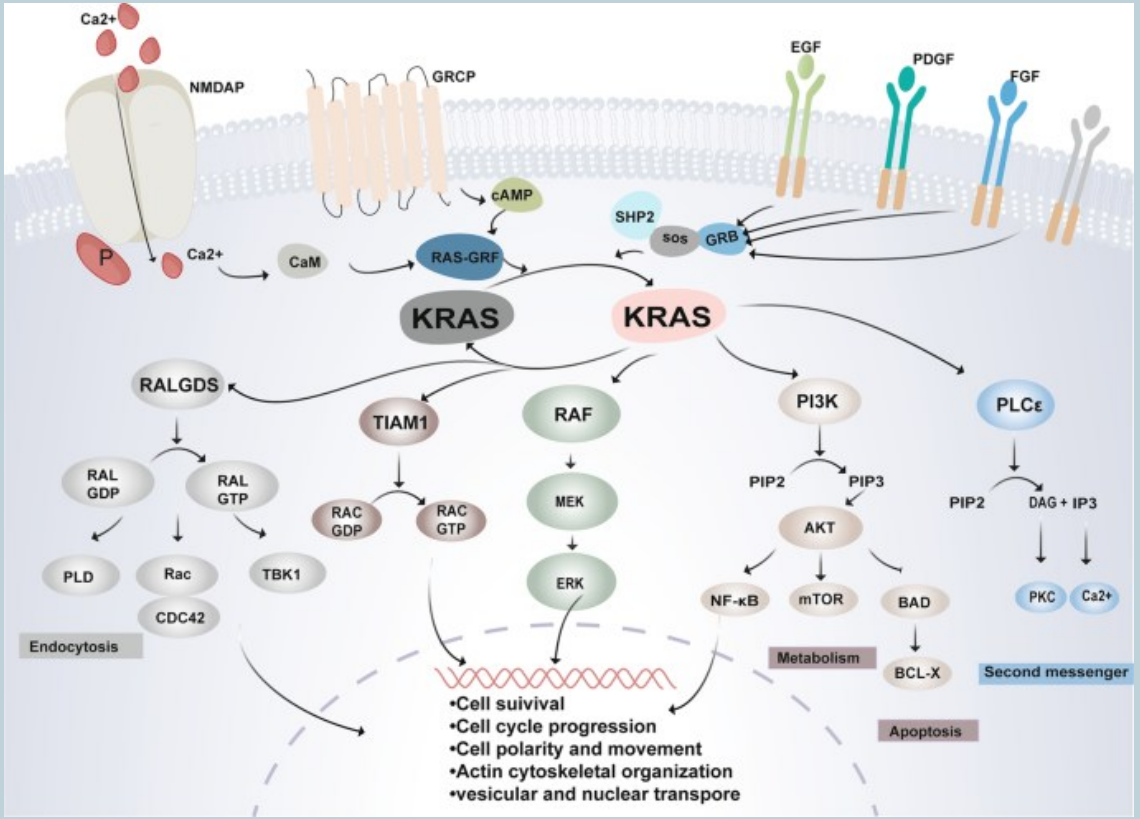
- 45 % CRC
- První známý onkogen (1982)
- Rodina RAS (HRAS, NRAS)
- Různé biologické chování dle mutace



Huang L et al, KRAS mutation: from undruggable to druggable in cancer, Signal Transduction and Targeted Therapy volume 6, Article number: 386 (2021)

KRAS

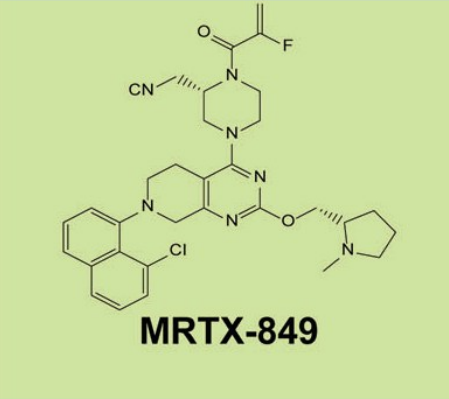
- G-protein s vazbou na buněčnou membránu (farnesylace)
- Přenos signálu uvnitř buňky signálními cestami:
  MAPK - proliferace
  PI3K - přežívání
  Alternativní aktivace (fúzní proteiny)
- Princip vypnuto/zapnuto - GDP/GTP

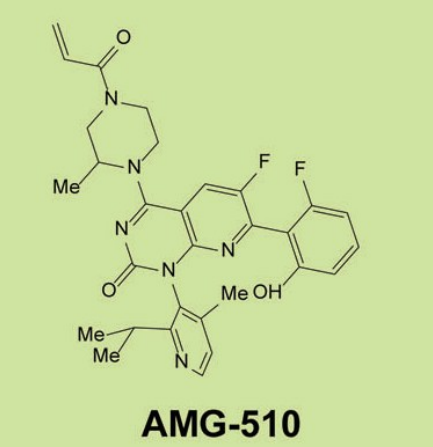


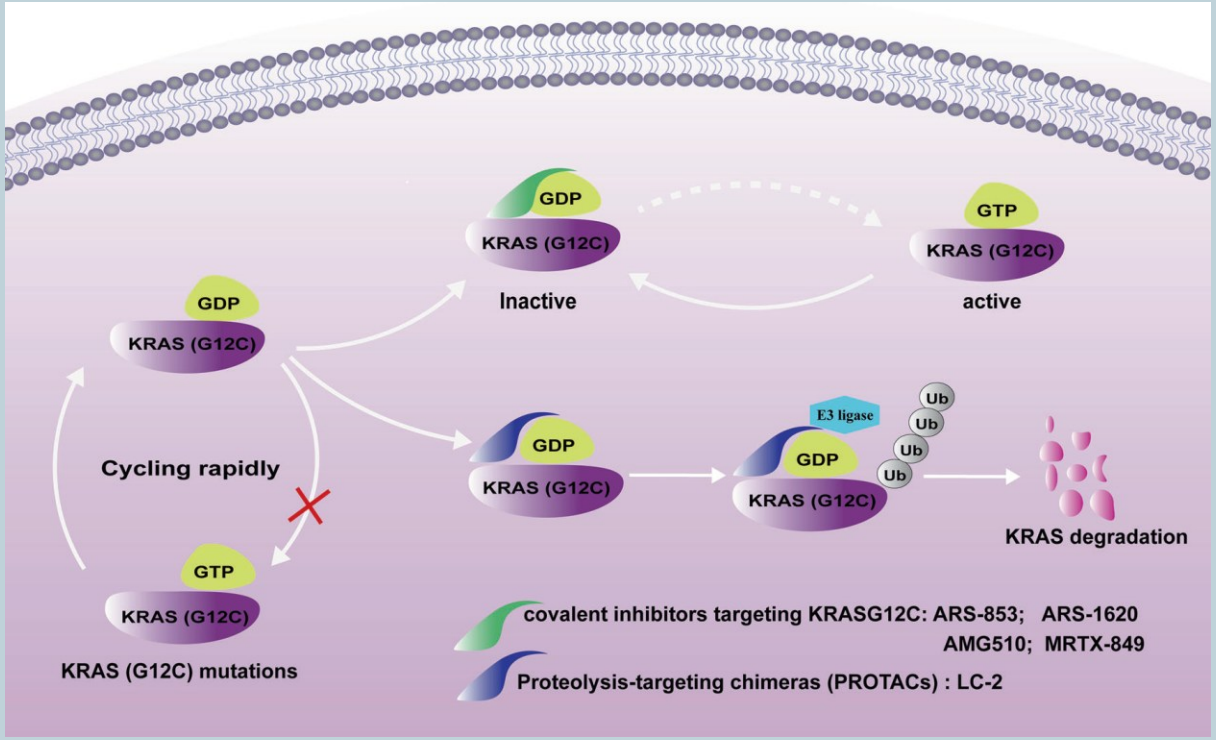
Burley SK et al, RCSB Protein Data Bank: Biological Macromolecular Structures Enabling Research and Education in Fundamental Biology, Biomedicine, Biotechnology and Energy, Nucleic Acid Research 47, 464-474 (2019)
Lamei Huang, Zhixing Guo, Fang Wang, Liwu Fu; KRAS mutation: from undruggable to druggable in cancer, Signal Transduction and Targeted Therapy volume 6, Article number: 386 (2021)

KRAS G12C

- 3-5 % CRC
- Cílení na disulfidický můstek Cys12 (blízko vazebnému místu pro GTP)
  - selektivita
  - větší aktivita (switch I, II region)
  - porušení interakce s efektorovými proteiny
  - degradace







Lamei Huang, Zhixing Guo, Fang Wang, Liwu Fu; KRAS mutation: from undruggable to druggable in cancer, Signal Transduction and Targeted Therapy volume 6, Article number: 386 (2021)

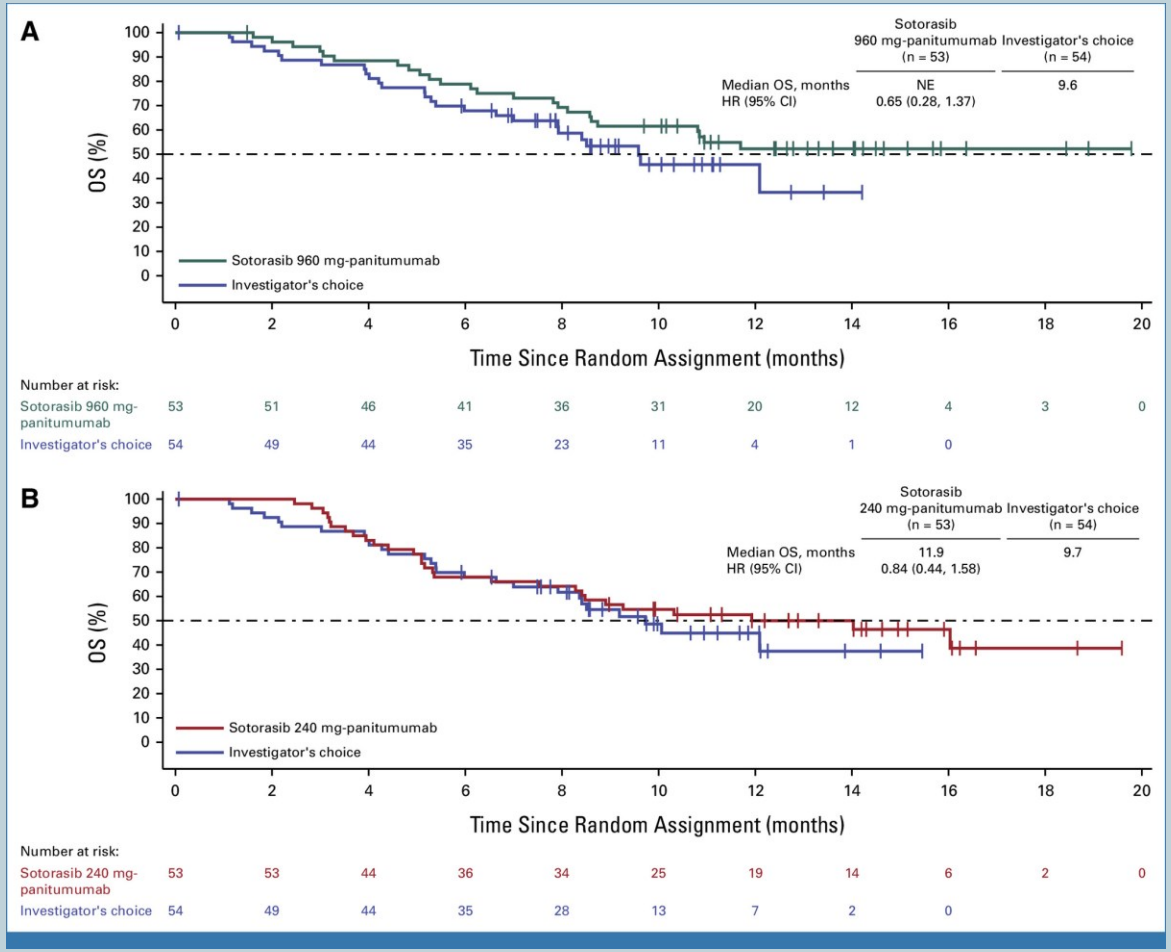
INHIBICE:

sotorasib + panitumumab (CodeBreaK 300)

mPFS (95% CI)
soto 960 + pani 5.8 M (4.2–7.5)
soto 240 + pani 4.0 M (3.7–5.9)
SOC 2.0 M (1.9–3.9),

mOS
soto 960 + pani NE (95% CI, 8.61-NE)
soto 240 + pani 11.9 M (95% CI, 7.52 to NE)
SOC 10.3 M (95% CI, 7.00 to NE)

ORR 30 %

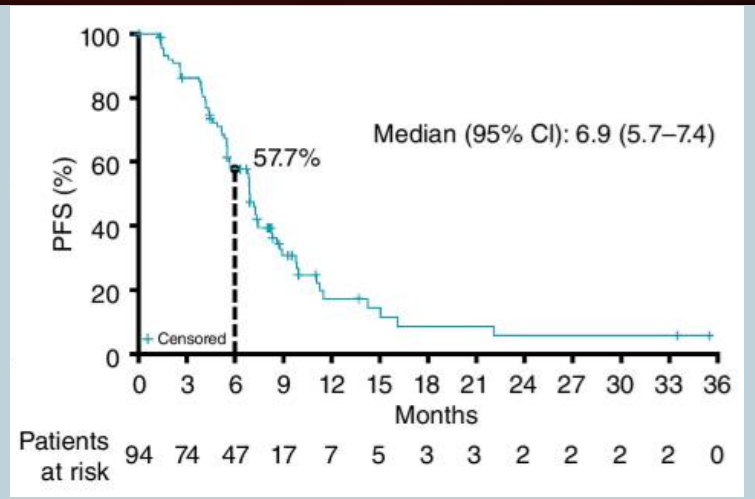


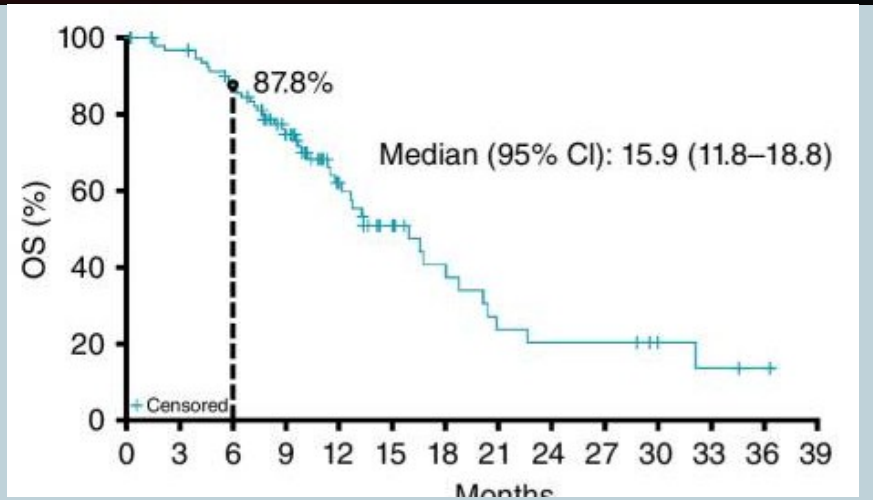
Fakih GM et al, Sotorasib plus Panitumumab in Refractory Colorectal Cancer with Mutated KRAS G12C, N Engl J Med 2023;389:2125-2139

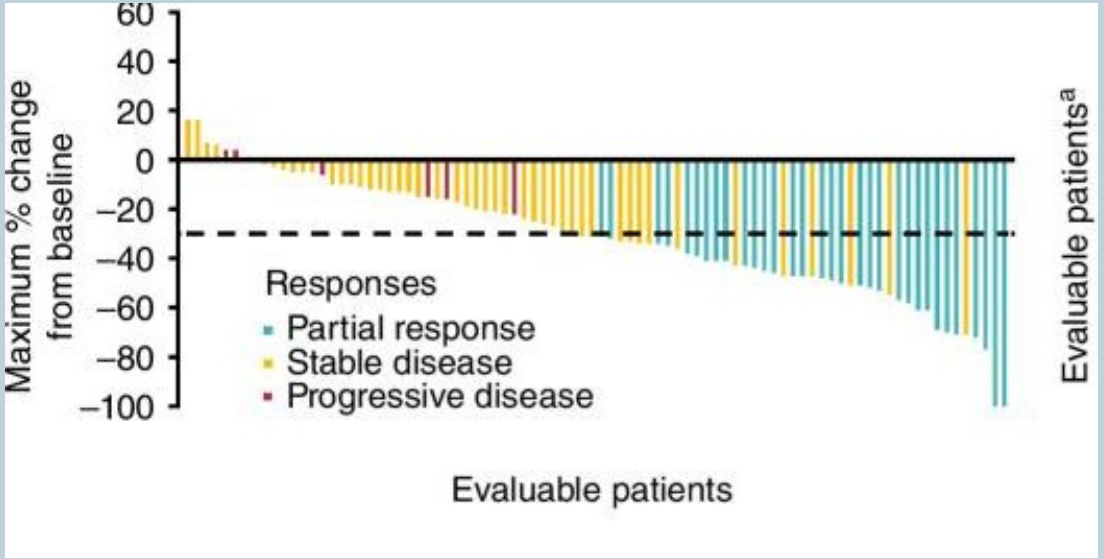
INHIBICE:

adagrasib + cetuximab (Krystal-1)

mPFS 6.9M (5.7–7.4)
mOS 15.9 M (11.8–18.8)
ORR 34.0 %





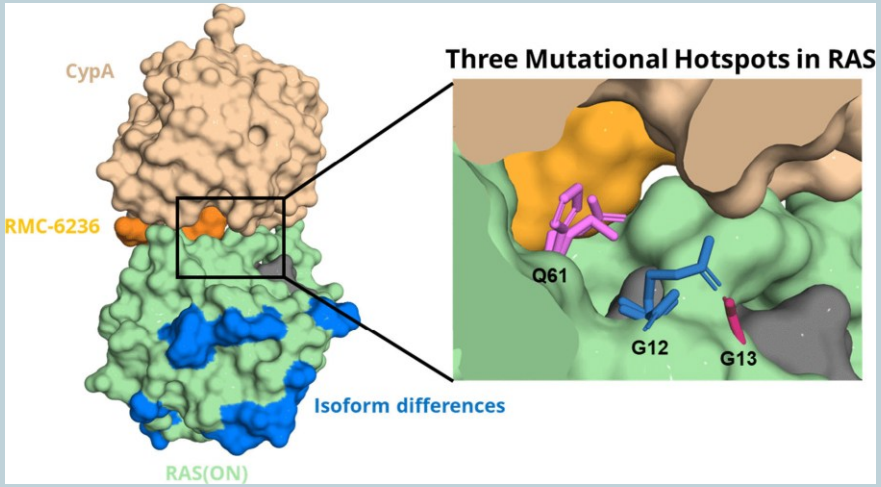


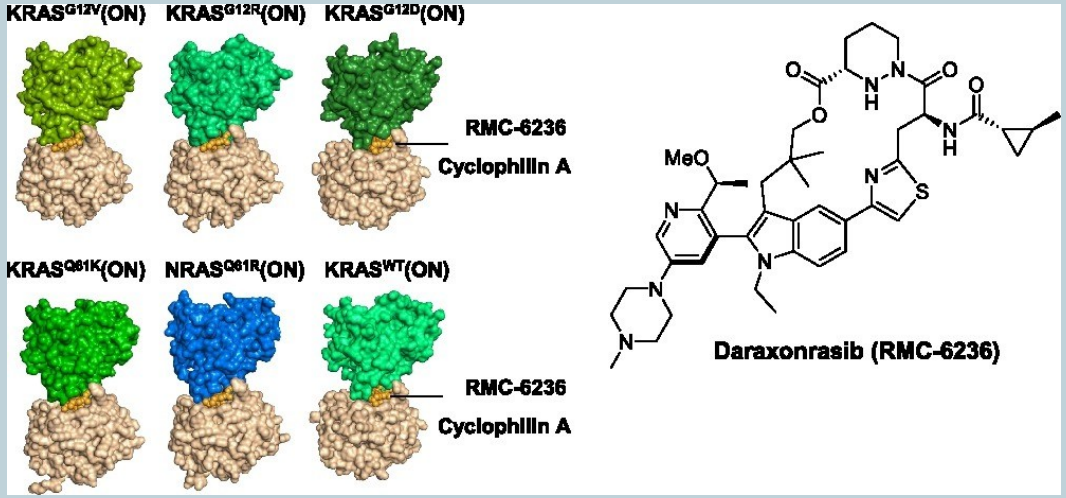
Yaeger R et al, Efficacy and Safety of Adagrasib plus Cetuximab in Patients with *KRAS*^{G12C}-Mutated Metastatic Colorectal Cancer,2024 Apr 8;14(6):982–993.

JINÉ non-G12C

- G12D
- panRAS (RMC-6236)

KRAS^{G12D} Inhibitor	Mechanism of Drug action		Clinical stage
HRS-4642	KRAS^{G12D} Inhibitor	HRS-4642 inhibits the binding of KRAS^{G12D} to SOS1 or RAF1, thereby inhibiting the downstream MEK-ERK signaling pathway	Phase I/II clinical study
MRTX1133	Non-covalently bonded KRAS^{G12D} selective inhibitor	MRTX1133 inhibits KRAS^{G12D} inactive and active states	Phase I clinical study
GFH375(VS-7375)	KRAS^{G12D} (ON/OFF) Inhibitor	GFH375 has a unique ON/OFF binding mechanism and can act on both activated (GTP-binding) and inactivated (GDP-binding) states of KRAS^{G12D} mutant protein. GFH375 binds to KRAS G12D protein in a non-covalent form, thereby inhibiting its binding to downstream effector proteins and suppressing their pathway activation, and ultimately inhibiting tumor cell proliferation to achieve anti-tumor effects.	Phase I/II clinical study
AST2169	KRAS^{G12D} Inhibitor (Liposome)	AST2169 liposome inhibits the function of KRAS G12D mutant protein, down-regulates the activity of related signaling pathways, effectively prevents cell cycle progression, induces apoptosis, and thus reduces the proliferation rate of tumor cells to achieve anti-tumor effects.	Phase I clinical study
RMC-9805	Covalent mutation-selective KRAS inhibitors	RMC-9805 selectively covalently modifies Asp-12 and interferes with RAS downstream signaling by forming a ternary complex with cyclophilin A (CypA) and the "ON" state of RASG12.	Phase I/II clinical study
ASP3082	KRAS^{G12D} protein degrader	ASP3082 is a novel small-molecule targeted protein degradation chimera that binds to and selectively targets KRAS^{G12D} mutant protein for degradation by recruiting an E3 ubiquitin ligase protein.	Phase I clinical study

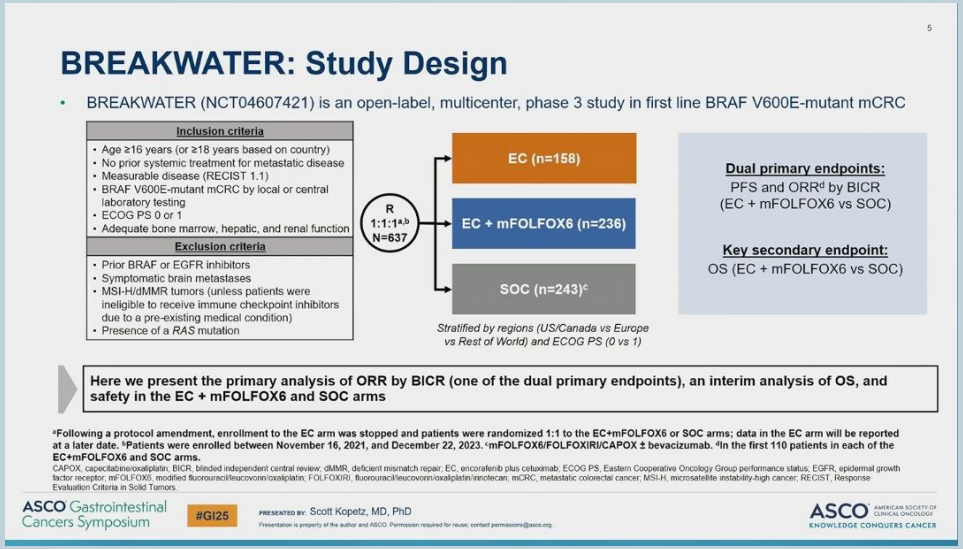


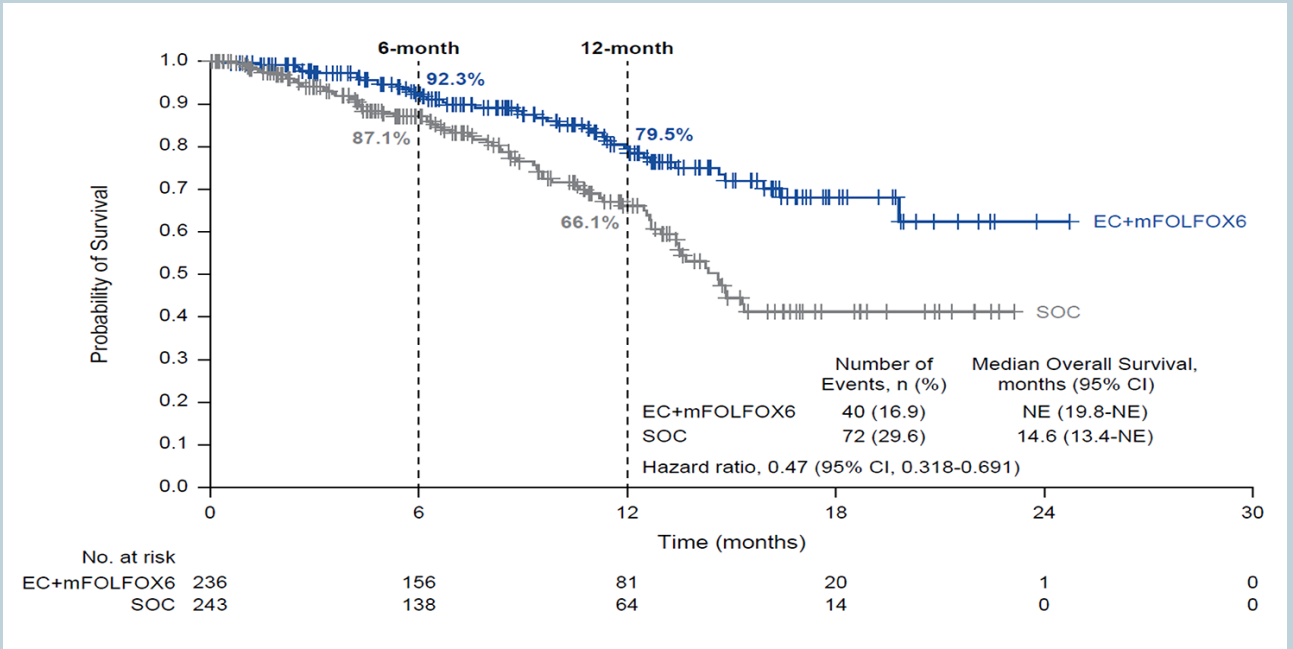


Li Y et al, New exploration of KRAS^{G12D} inhibitors and the mechanisms of resistence, Experimental Hematology & Oncology volume 14, Article number: 39 (2025), Cregg et al, Discovery of Daraxonrasib (RMC-6236), a Potent and Orally Bioavailable RAS(ON) Multi-selective, Noncovalent Tri-complex Inhibitor for the Treatment of Patients with Multiple RAS-Addicted Cancers, Journal of Medicinal Chemistry Available online 10 March 2025

BRAF V600E

- 8-12 % CRC
- Breakwater:
  encorafenib + cetuximab + mFOLFOX6
  vs. CAPOX, FOLFOX, FOLFOXIRI +/- bevacizumab

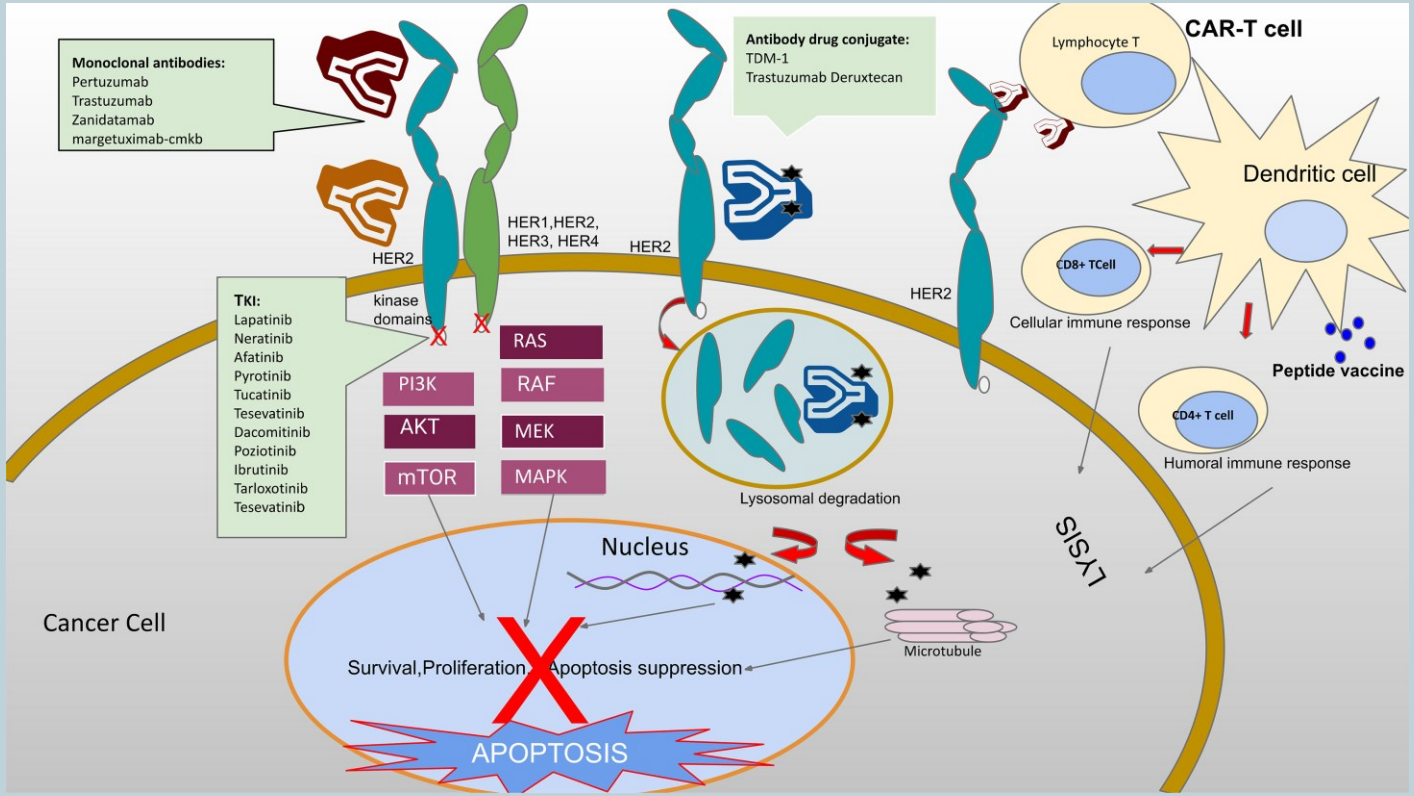




Kopetz, S., Yoshino, T., Van Cutsem, E. *et al.* Encorafenib, cetuximab and chemotherapy in BRAF-mutant colorectal cancer: a randomized phase 3 trial. *Nat Med* **31**, 901–908 (2025).

HER2/neu

- amplifikace 3-5 % CRC
- častěji KRAS/BRAF-wt
- resistence k EGFRi
- častěji mozková meta
- mutace?
  - aktivační S310F, L755S, V777L, V842I



Djaballah et al, HER2 in Colorectal Cancer: The Long and Winding Road From Negative Predictive Factor to Positive Actionable Target, American Society of Clinical Oncology Educational Book Volume 42, 2022

HER2/neu

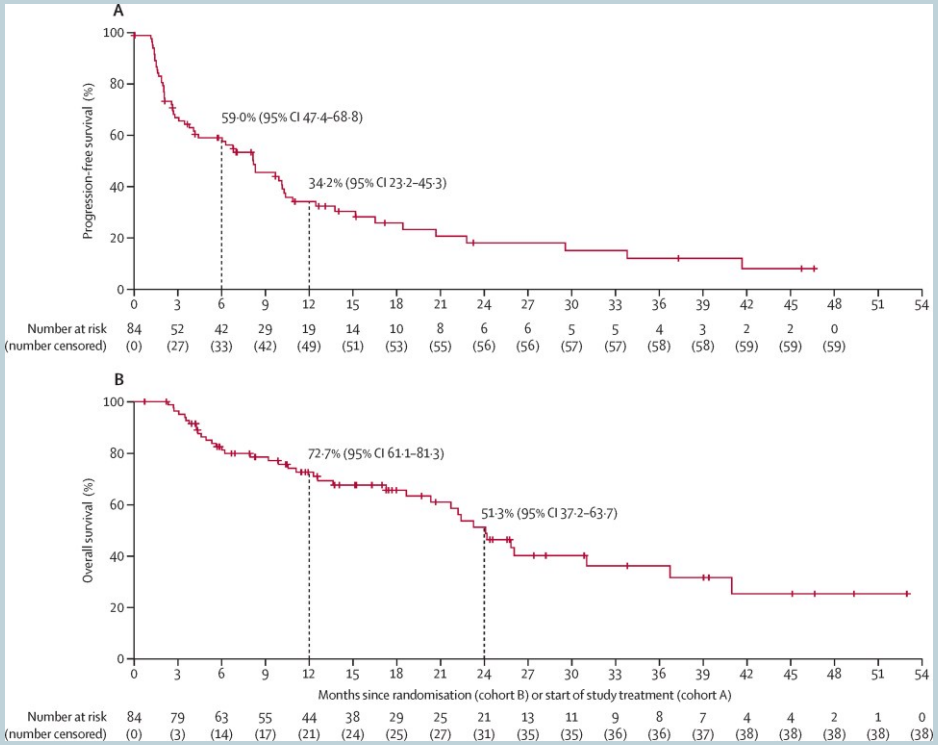
Trial name	Phase	Median number of prior therapy	Experimental regimen	Overall ORR, % (95% CI)	Overall DOR	Median OS (95% CI)	Subgroup analysis based on IHC score	Notable side effects
	mAb							
MyPathway[20]	II	4	Trastuzumab plus pertuzumab	32% (20–45)	5.9 months	Not reported	Not reported	Ventricular dysfunction (2%)
TAPUR[32]	II	Not reported (77% received 1 to 2 prior lines of therapy)	Trastuzumab plus pertuzumab	25% (CI 11–45)	Not reported	60 weeks	Not reported	Ventricular dysfunction (1%)
Meric-Bernstam *et al.*[26]	I	4	Zanidatamab	38% (20–59)	6.3 months (in the dose escalation CRC group)	NA	NA	Diarrhea (all grades, 43–52%), arthralgia (2%), hypophosphatemia (2%)
	TKI-containing regimens							
HERACLES-A[33]	II	5	Trastuzumab plus lapatinib	30% (CI 14–50)	4.7 months	46 weeks (33–68)	Not reported	Diarrhea (all grades, 78%)
MOUNTAINEER[11,34]	II	3 in doublet arm 2 in tucatinib alone arm	Tucatinib with or without trastuzumab	Tucatinib plus trastuzumab: 38.1% (95% CI not reported) Tucatinib alone: 3.3% (0.1–17.2) Tucatinib cross-over: 17.9% (6.1–36.9)	12.4 months	Not reported	ORR in HER2 IHC2+/FISH+ group: 20% ORR in IHC 3+ group: 41.1–46.7%.	Diarrhea (64% in the double arm, 33% in the tucatinib alone arm), acute kidney injury, elevated liver enzymes
HER2-FUSCC-G[35]	IIa	Not reported (inclusion criteria includes at least 2 prior lines of treatment)	Pyrotinib plus trastuzumab	57.1% (95% CI not reported)	Not reported	Not reached	Not reported	Diarrhea (grade 3, 25%), rash (grade 3, 6.3%)

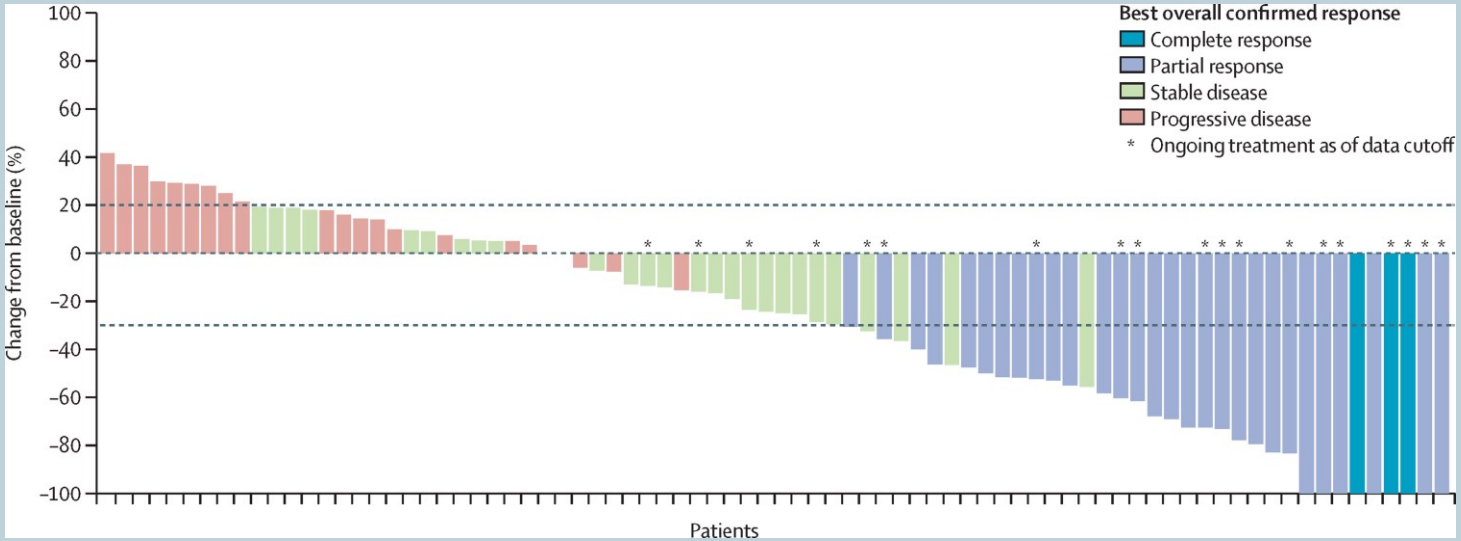
HER2/neu

	ADC							
DESTINY-CRC01[10]	II	4	Fam-trastuzumab deruxtecan	Cohort A (ICH3+ and 2+/FISH positive): 45.3% (31.6–59.6)	7 months	Cohort A: 5.4 months (95% CI not reported)	ORR in the ICH3+ group: 57.5% (40.9–73) ORR in ICH2+/FISH positive group: 7.7% (CI 0.2–36)	Pneumonitis (all grades, 6%, which included two cases of grade 2, one grade 3, and two grade 5)
DESTINY-CRC02[36]	II	5.4 mg/kg T-DXd group: 3 6.4 mg/kg T-DXd group: 4	Fam-trastuzumab deruxtecan	5.4 mg/kg T-DXd group: 37.8% 6.4 mg/kg T-DXd group: 27.5%	5.5 months	NA	IHC3+ group that received 5.4 mg/kg T-DXd: 46.9% IHC2+/FISH+ group that received 5.4 mg/kg T-DXd: 5.6% IHC3+ group that received 6.4 mg/kg T-DXd: 29.4% IHC2+/FISH+ group that received 6.4 mg/kg T-DXd: 16.7%	Pneumonitis (8.4% with 5.4 mg/kg T-DXd, 12.8% with 6.4 mg/kg T-DXd)

HER2/neu

- Tucatinib + trastuzumab (Mountaineer)



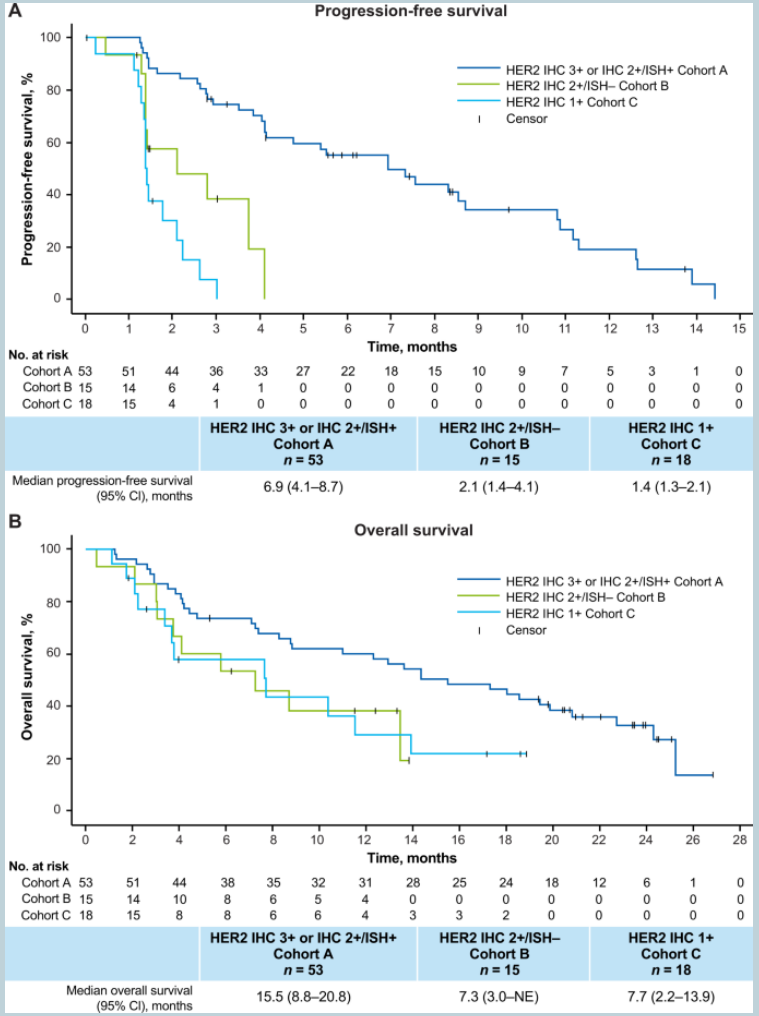


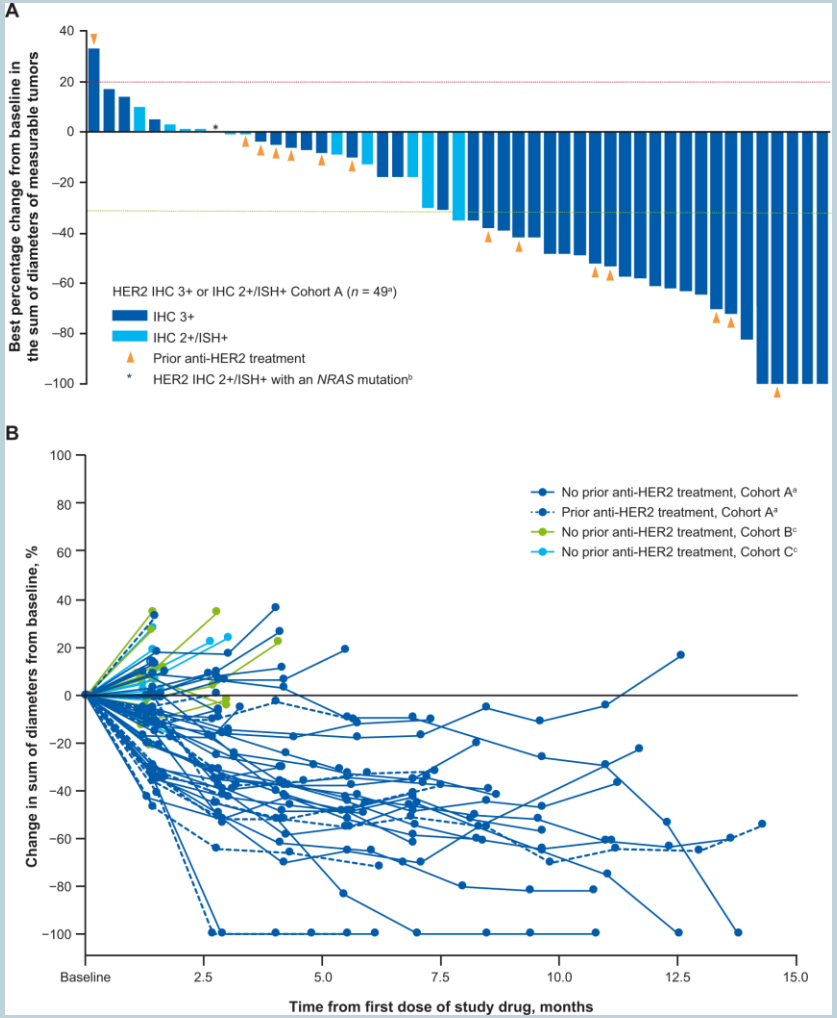
Strickler et al, Tucatinib plus trastuzumab for chemotherapy-refractory, HER2-positive, RAS wild-type unresectable or metastatic colorectal cancer (MOUNTAINEER): a multicentre, open-label, phase 2 study, Lancet 24, Issue 5p496-508May 2023

HER2/neu

- Trastuzumab-deruxtecan (Destiny-CRC-01)
  - amplifikace
  - podskupina s mutací HER ORR <10 %

(obdobně trastu/pertu ve studii MyPathway)

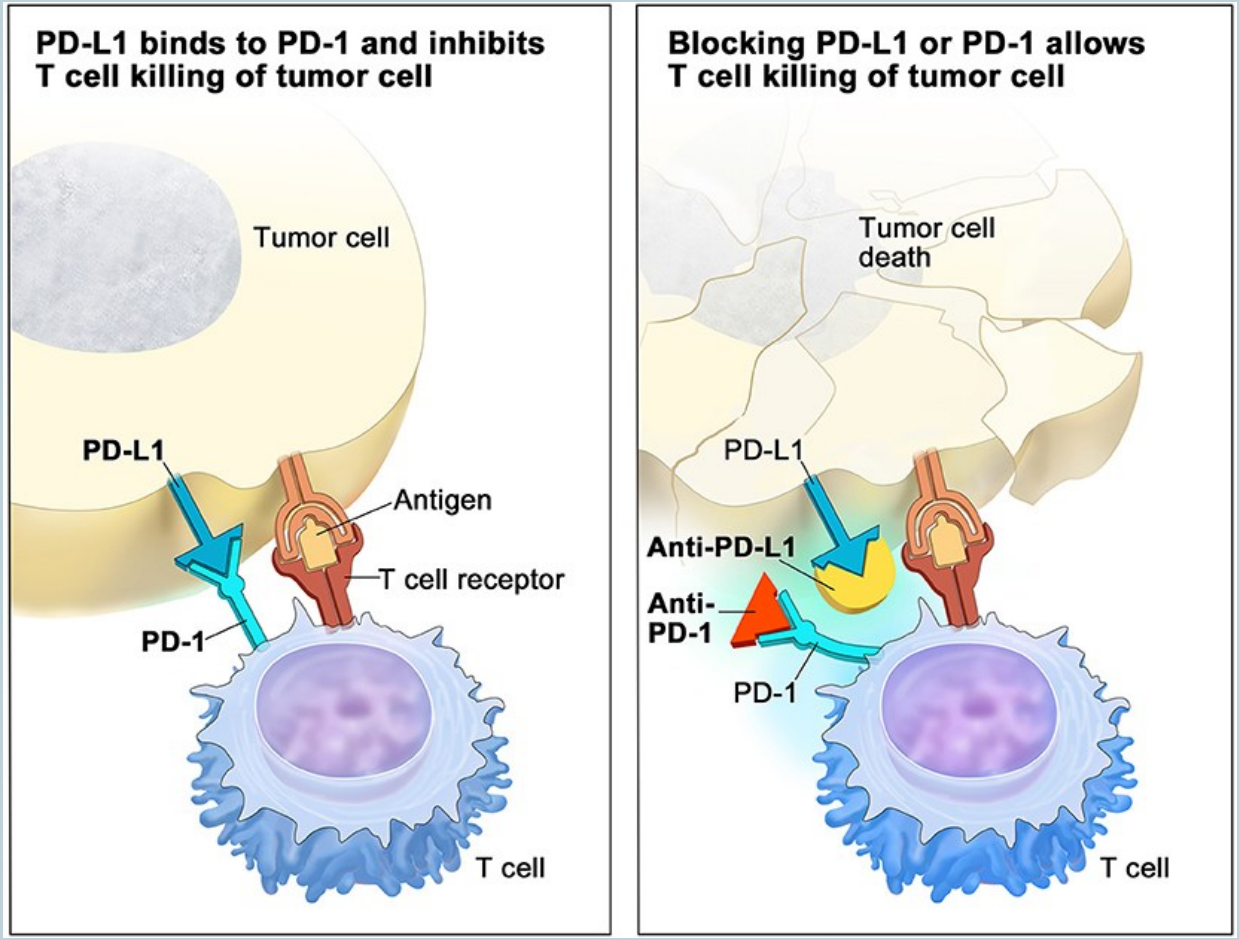




Yoshino T et al, Final results of DESTINY-CRC01 investigating trastuzumab deruxtecan in patients with HER2-expressing metastatic colorectal cancer, Nature Communications volume 14, Article number: 3332 (2023)

IMUNOTERAPIE - PDL-1

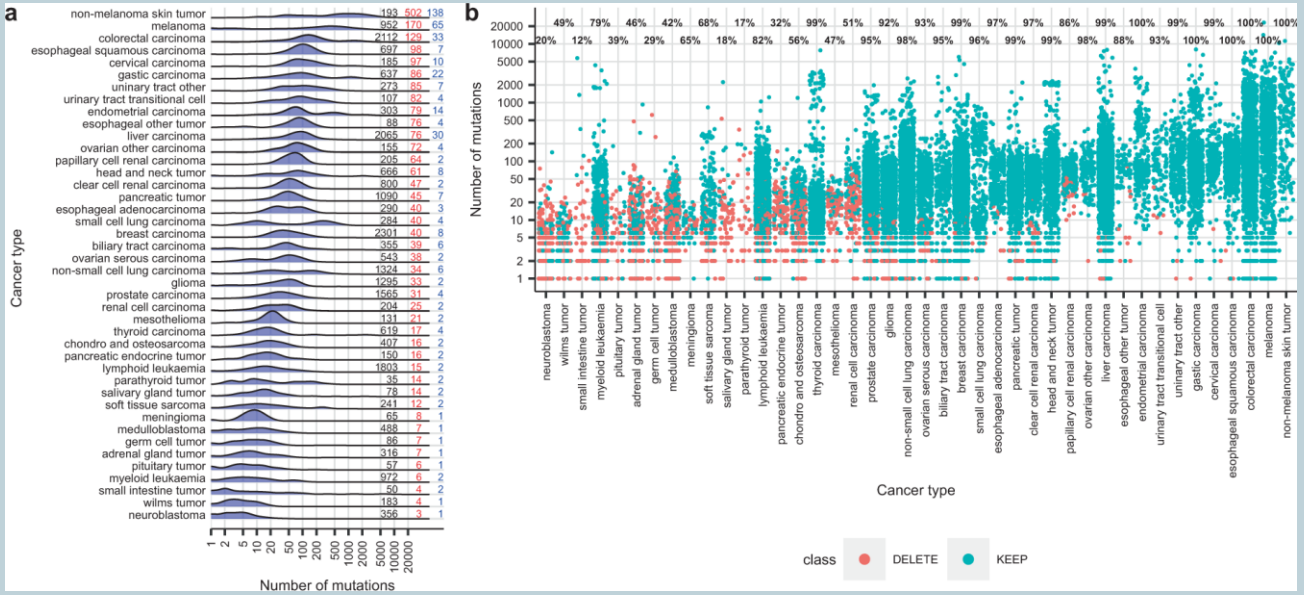
- velmi variabilní exprese
- ↑ exprese PD-L1 je spojena s horší prognózou v rámci MSI-H CRC
- potenciálně prognostický a prediktivní charakter
- standardní využití u CRC chybí



Rosenbaum et al, PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumor-infiltrating lymphocytes, Mod Pat 29, 1104–1112, 2016
Oliveira et al, Review of PD-1/PD-L1 Inhibitors in Metastatic dMMR/MSI-H Colorectal Cancer, Front Oncol 14:9:396, 2019

IMUNOTERAPIE - TMB

- potenciálně prediktivní marker imunoterapie
- počet somatických mutací v genomu (vyjádřeno megabázi gen. materiálu)
- různé hranice (10, 17, 20 mut/Mb), chybí standardizace (jaké mutace jsou počítány? z čeho? vzorky?)



Marques et al, Tumor mutational burden in colorectal cancer: Implications for treatment, Critical Reviews in Oncology / Hematology 197, 104342, 2024
Martínez-Pérez et al, Panels and models for accurate prediction of tumor mutation burden in tumor samples, NPJ Prec Onc 5, Article number: 31, 2021

IMUNOTERAPIE - MSI/TMB/MMR

- bez hypermetylace promotoru MLH1, bez mutace EPCAM
- (15 %) pacientů s diskordantními výsledky
  - ztráta MMR bez mikrosatelitové nestability
  - zachovale MMR s MSI
- není korelace s TMB

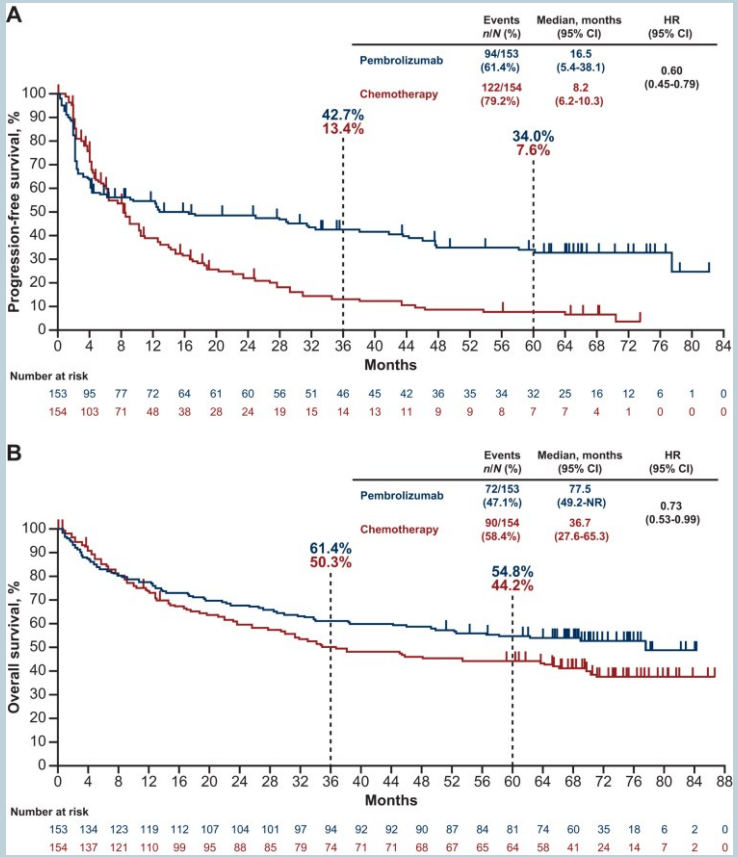
Jaffrelot M et al, An unusual pehnotype occurs in 15% of MMR-d tumors and is associated with non-colorectal cancers and genetic syndromes, Modern Pathology 35, 427-437 (2022)

IMUNOTERAPIE CRC

- PD(L)-1 inhibice, v kombinaci anti-CTLA-4
  - pembrolizumab, nivolumab + nivolumab, dostarlimab
- zatím jediné prediktivní markery:

dMMR/MSI-H, POLE-mut (POL-D1)

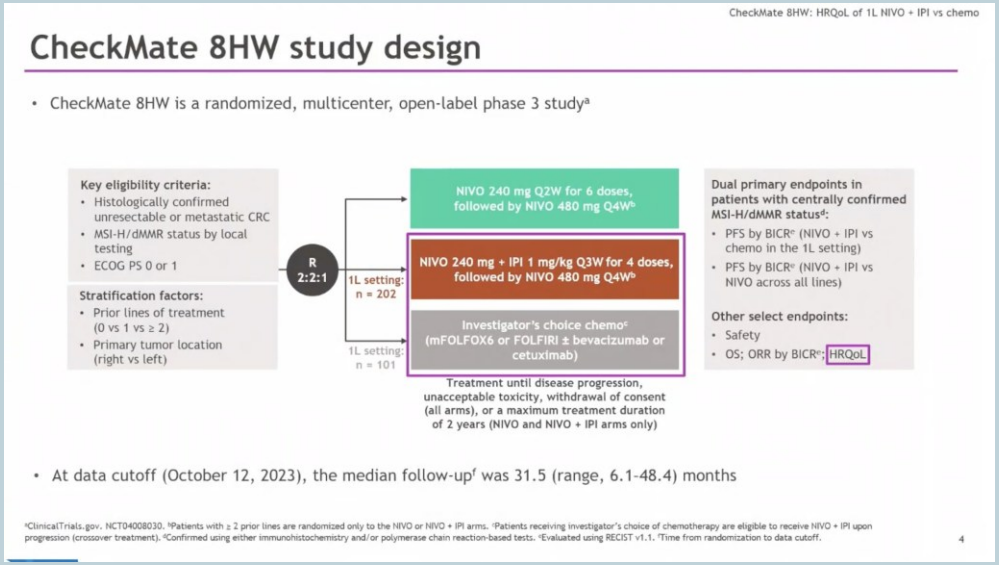
- pembrolizumab (Keynote-177)

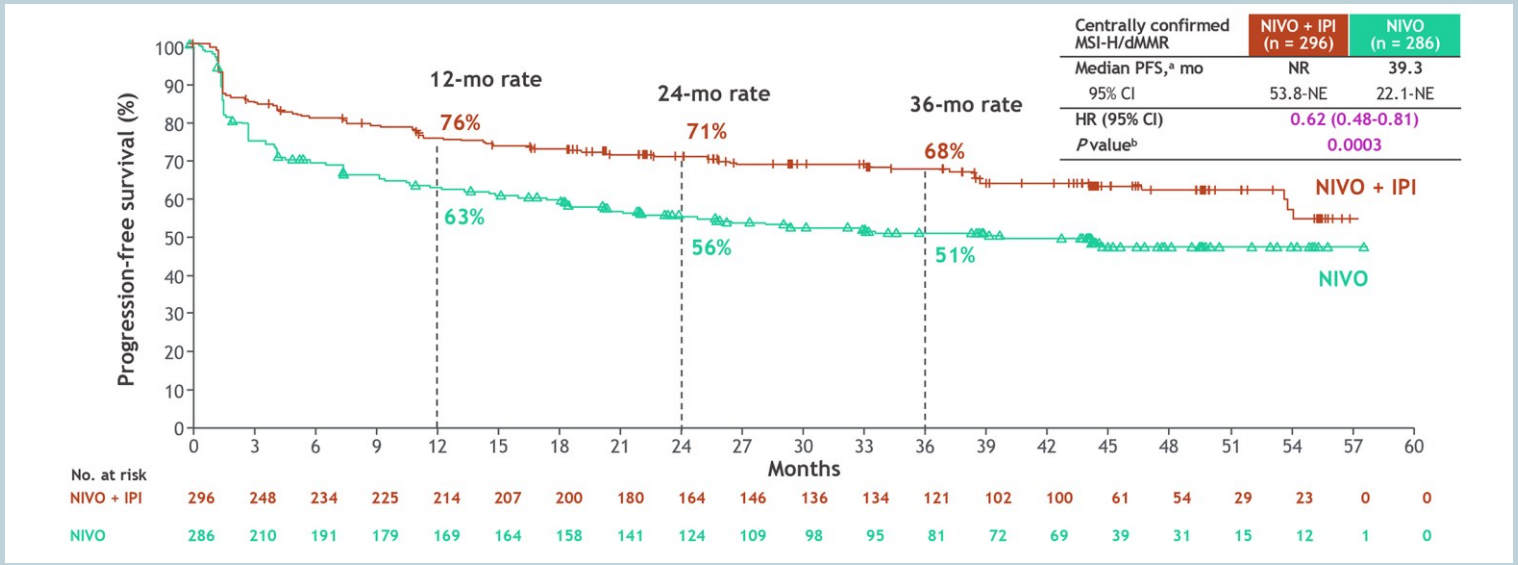


André T et al, Pembrolizumab in Microsatellite-Instability–High Advanced Colorectal Cancer, N Engl J Med 2020;383:2207-2218

IMUNOTERAPIE CRC

- ipilimumab + nivolumab (Checkmate)

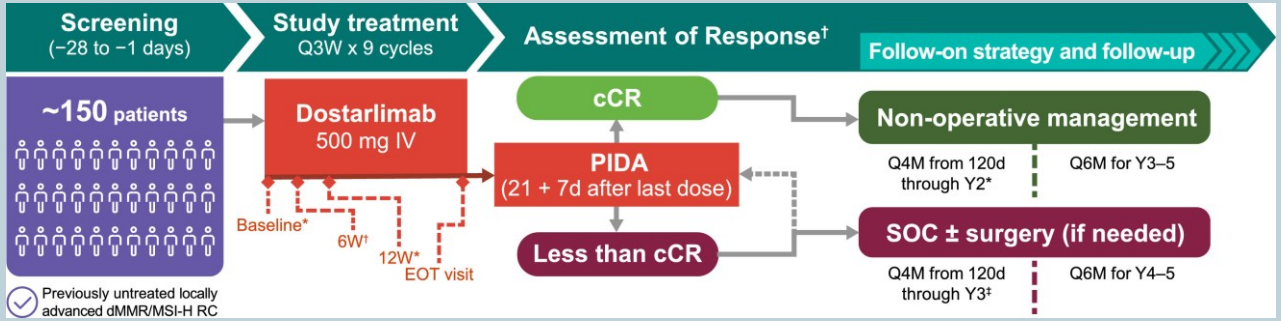


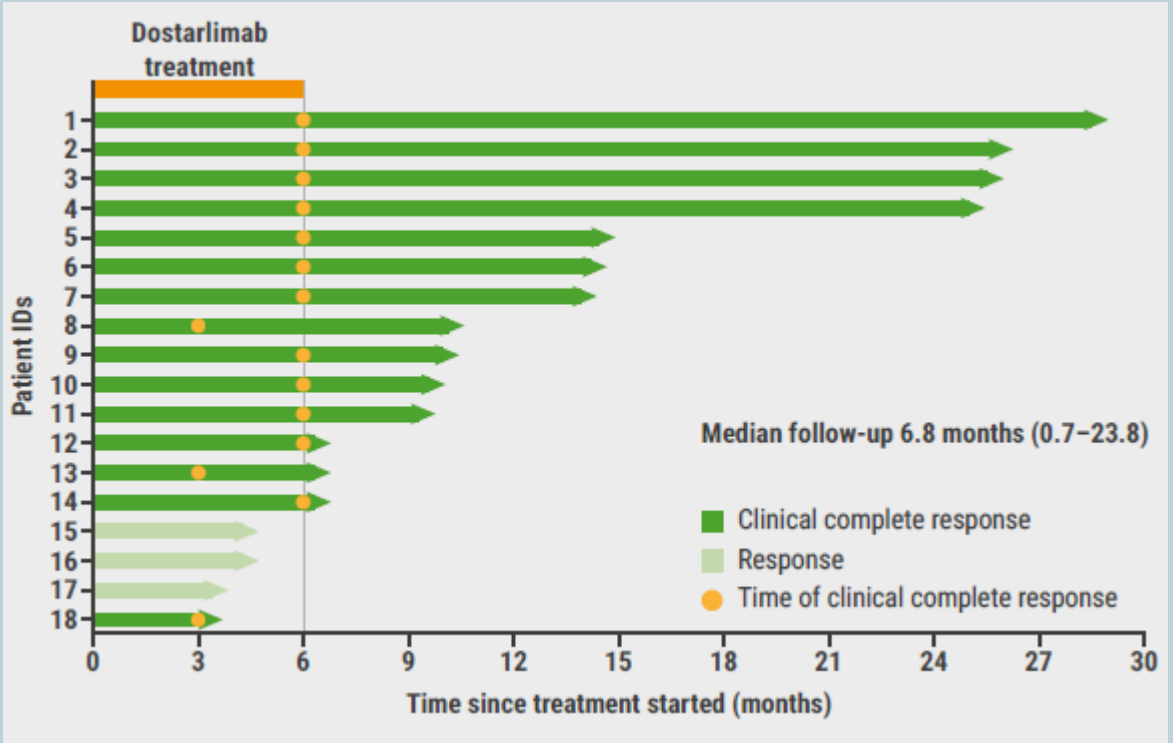


André T et al, Nivolumab plus Ipilimumab in Microsatellite-Instability–High Metastatic Colorectal Cancer, NEJM 391(21):2014-2026 (2024)

IMUNOTERAPIE

- dostarlimab (Azur-1,2)
  - dMMR
  - ORR 100 %,

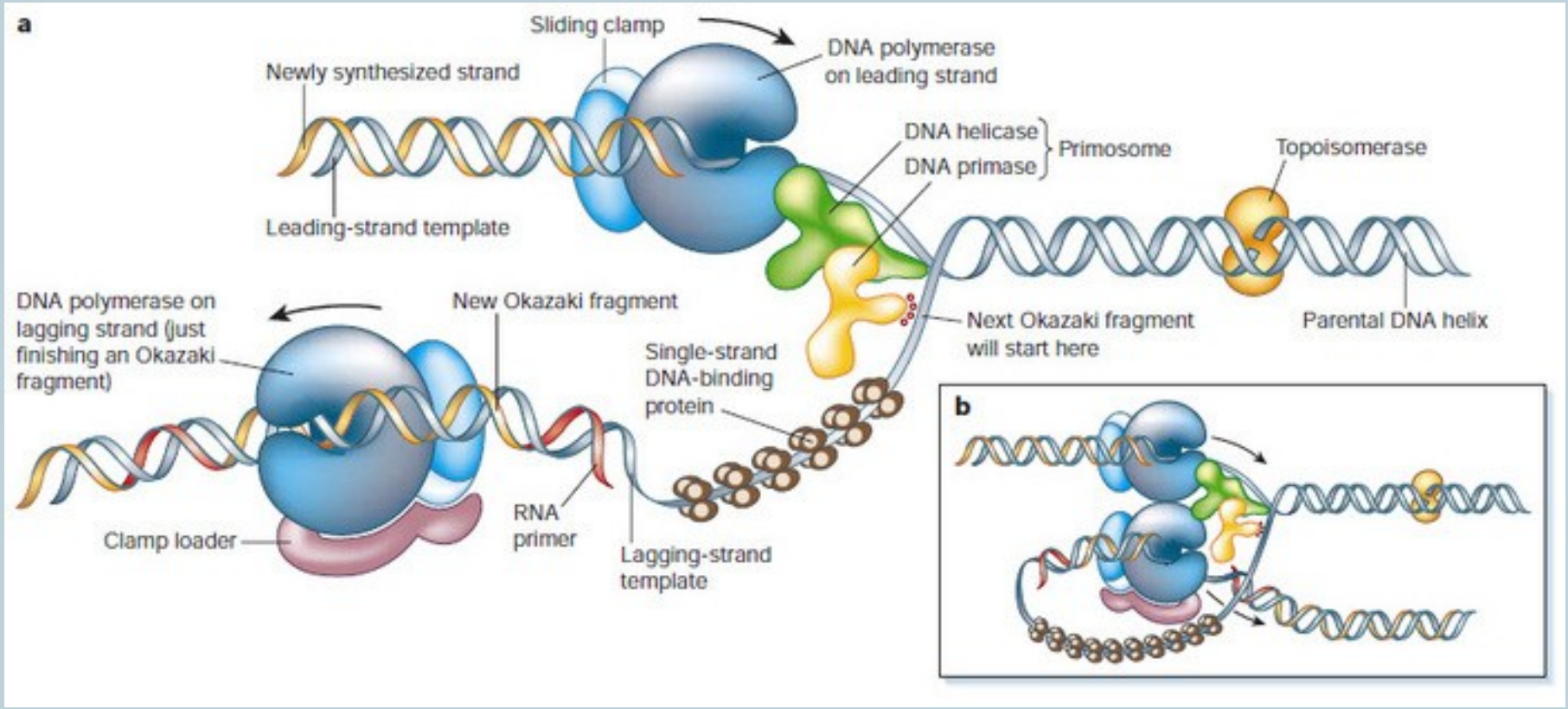




Cercek et al, A Phase Two, Single-Arm, Open-Label Study With Dostarlimab Monotherapy in Participants With Untreated Stage II/III dMMR/MSI-H Locally Advanced Rectal Cancer (AZUR-1), Clinical Colorectal Cancer, 24, 2, 2025, 325-330

POLE

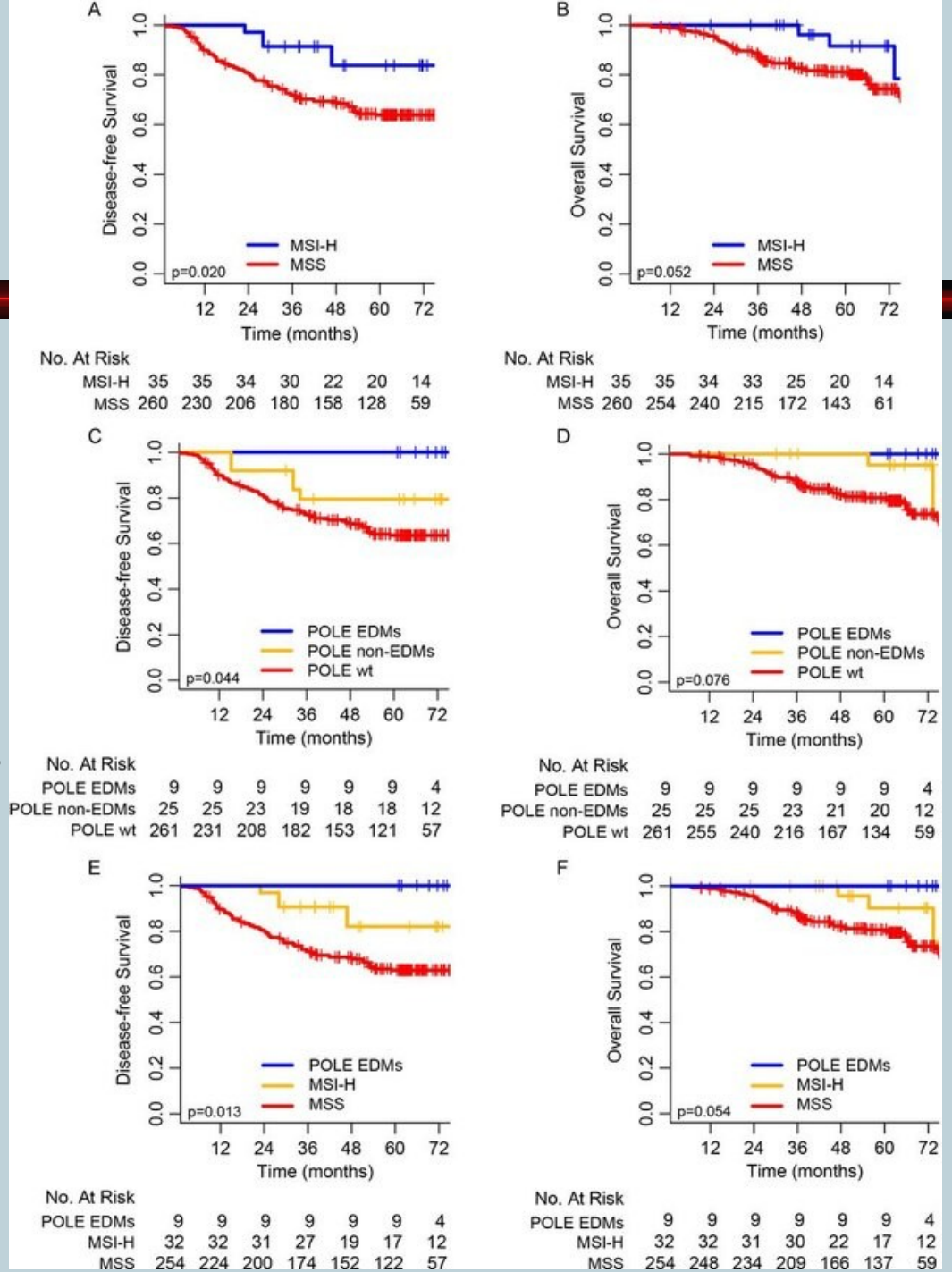
- u člověka 15 typů polymeráz (rodina A, B, X, Y)



Ghosh et al, 20 years of DNA Polymerase μ, the polymerase that still surprises, FEBS Journal 288, 7230–7242 , 2021
Das-Bradoo, S. & Bielinsky, A. (2010) DNA Replication and Checkpoint Control in S Phase. Nature Education 3(9):50, 2010

POLE

- spojeno s tzv. hypermutovaný fenotypem (TMB 50 mut/Mb a více)
- ↑ přítomnost neoantigenů
- ↑ tumor-infiltrující lymfocyty
- častěji mladší muži, pravostranné tumory, zřídka KRAS-mut
- lepší prognóza (za podmínky mutace v exonukleázové doméně a TMB-high)

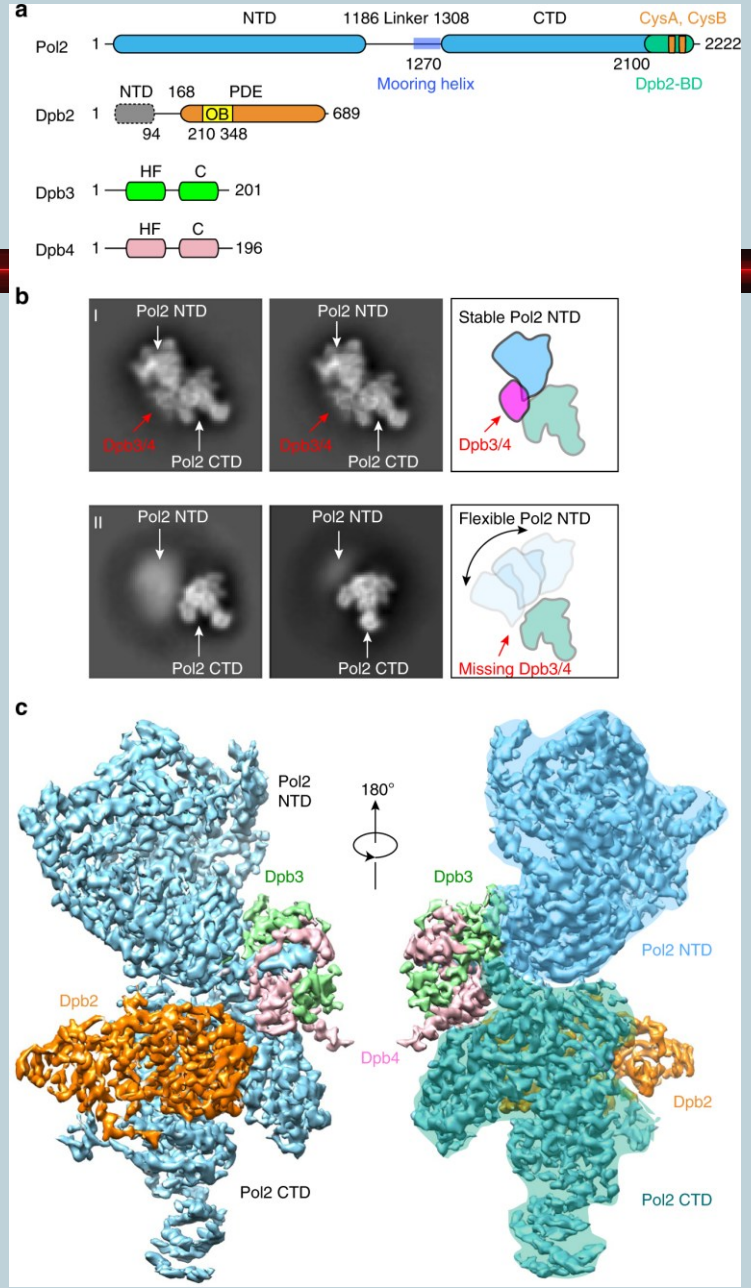


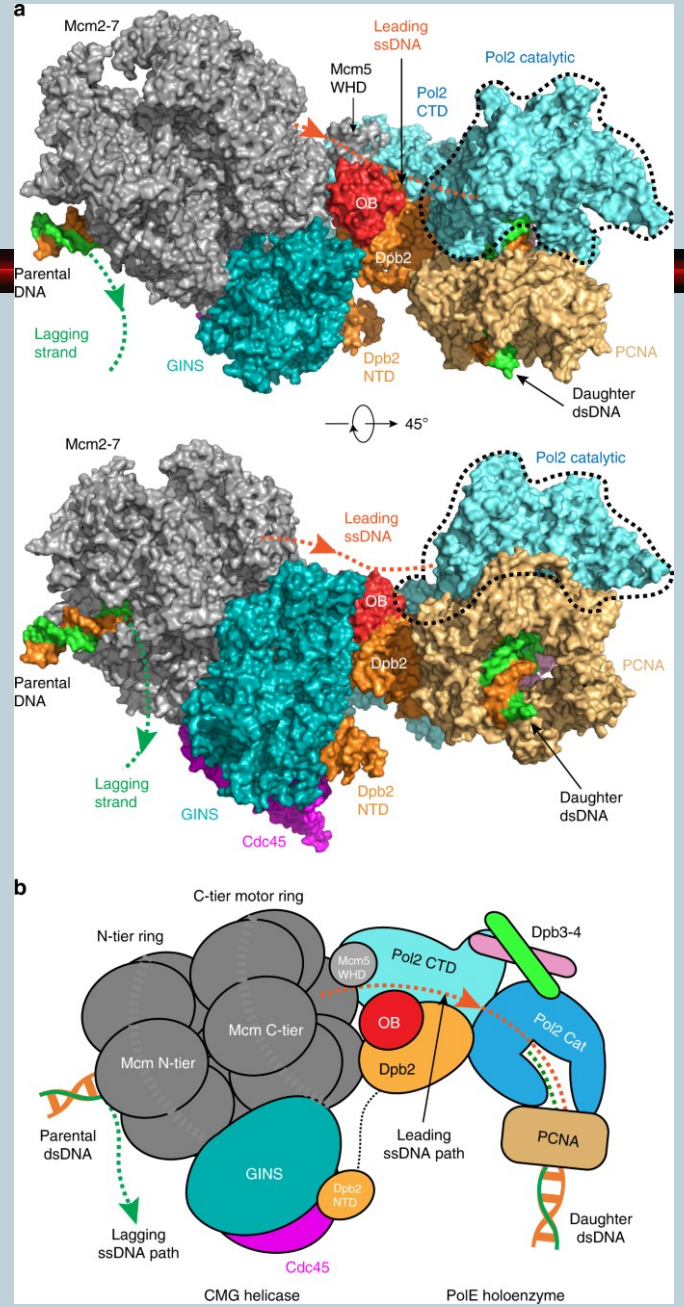
POLE

- rodina polymeráz B, multimerická (POLE 1-4)
  - replikace chromozomů (elongace vedoucího řetězce)
  - NER (nucleotide excision repair)
  - mismatch repair (proofreading)
- mutace v exonukleázové doméně vedou k poruše její funkce a zvýšení počtu SNV (single nucleotide variants)
- somatické mutace 2-8 % CRC (pMMR, MSS)
- zárodečné mutace POLE (polymerase proofreading-associated polyposis) spojeny s adenomy a adenokarcinomy tlustého střeva
- další souvislost s endometroidním karcinomem (vyšší frekvence)

POLE

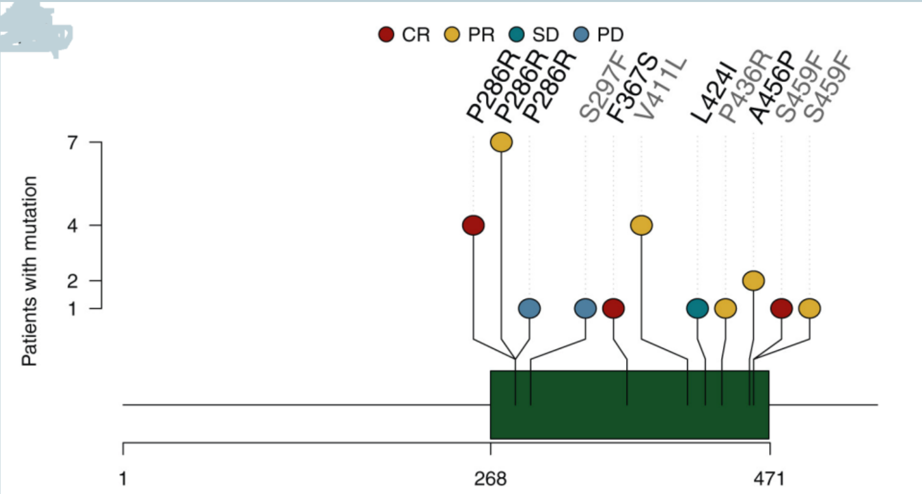
- 4 podjednotky
- úzké napojení na ostatní proteiny (PCNA, CMG helikáza, ...)
- opravy DNA

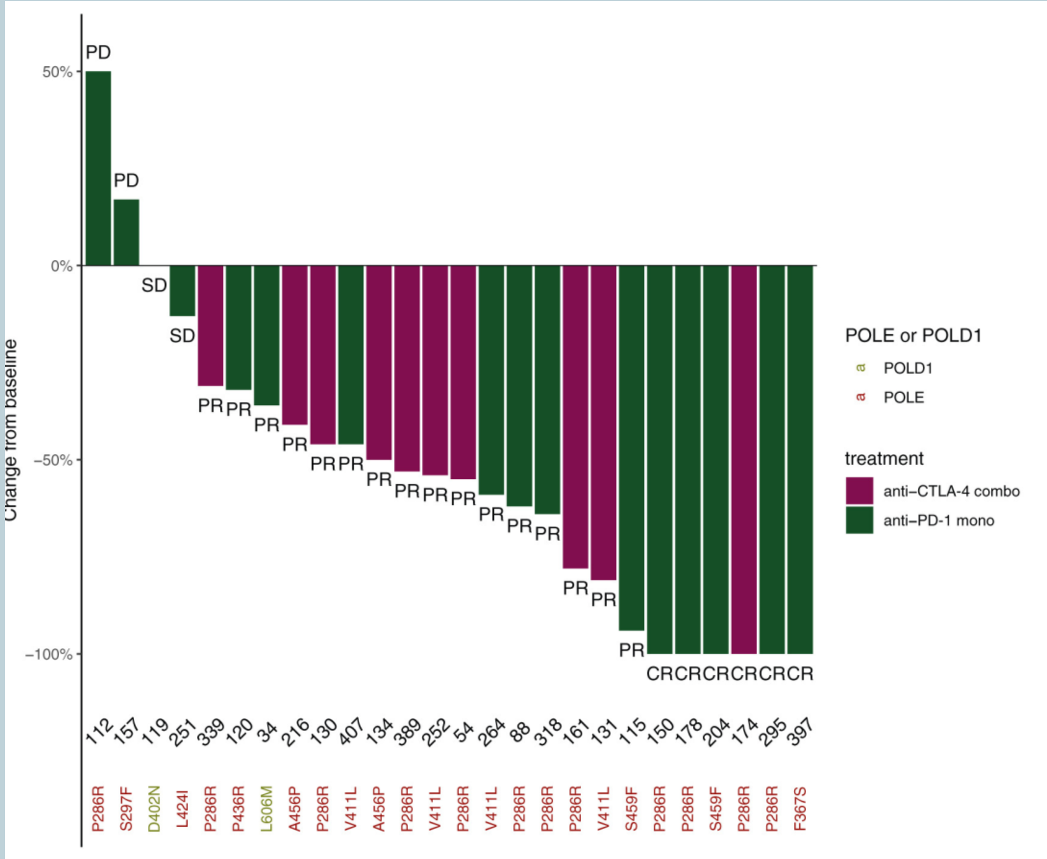




POLE

- lepší odpovídavost na imunoterapii i ve srovnání s dMMR/MSI-H CRC
- nejčastější mutace POLE
  sporadické: P286R, V411L, P286H/S, F367S, S459F
  germinální: V424L





Ambrosini et al, Immune checkpoint inhibitors for POLE or POLD1 proofreading-deficient metastatic colorectal cancer, Annals of Oncology 35(7),p643-655, 2024

POLE

- POLE je další z terapeutických cílů umožňující účinné podání imunoterapie
- nutnost detailního testování (napříč diagnosami);
- přetrvávají nežádoucí účinky spojené s imunoterapií

ZÁVĚR

- molekulární testování může přinést výsledky v každé fázi léčby CRC
- volba vhodného postupu by měla být závislá na rozhodnutí tumor-boardu