2025 ESMO IMMUNO-ONCOLOGY
Annual Congress

The New Era of CTLA-4 Modulators

Special Session with Industry: Early Pipelines of Biotech Companies

Steven J. O'Day, MD
Chief Medical Officer, Agenus Inc.

December 11, 2025

DECLARATION OF INTERESTS

Leadership role and stock ownership at Agenus Inc.

Immuno-Oncology Before the Checkpoint Revolution

- **Activity limited to highly immunogenic cancers**[1,2]
  i.e., melanoma and RCC

- **High-dose cytokines (IL-2, IFN-α)**[2,3]
  Rare durable remissions; substantial toxicity

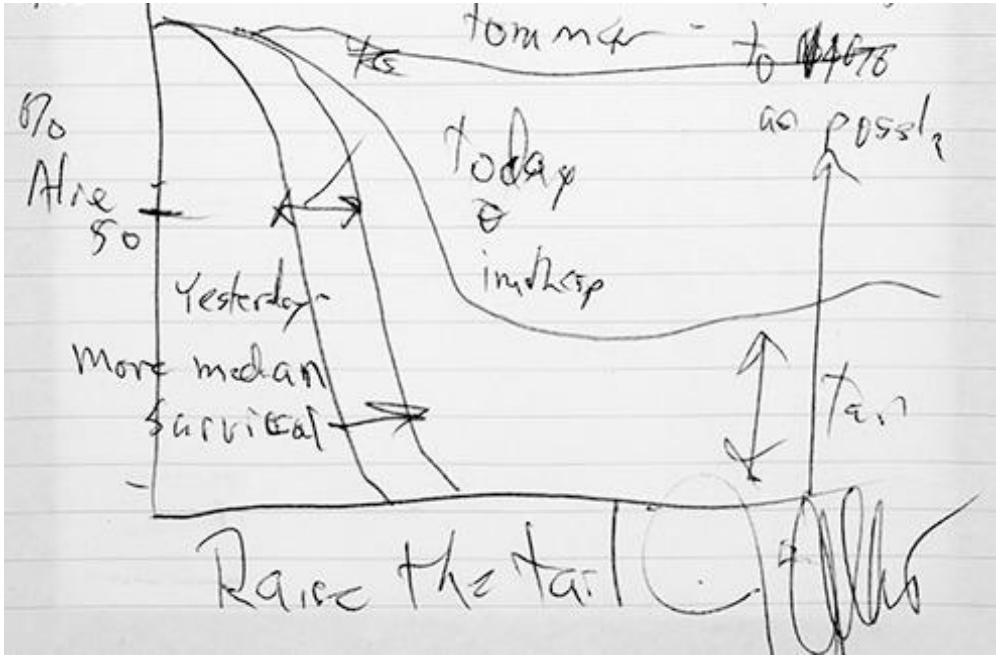
- **Cancer vaccines**[1]
  Strong biologic rationale; clinical benefit not broad

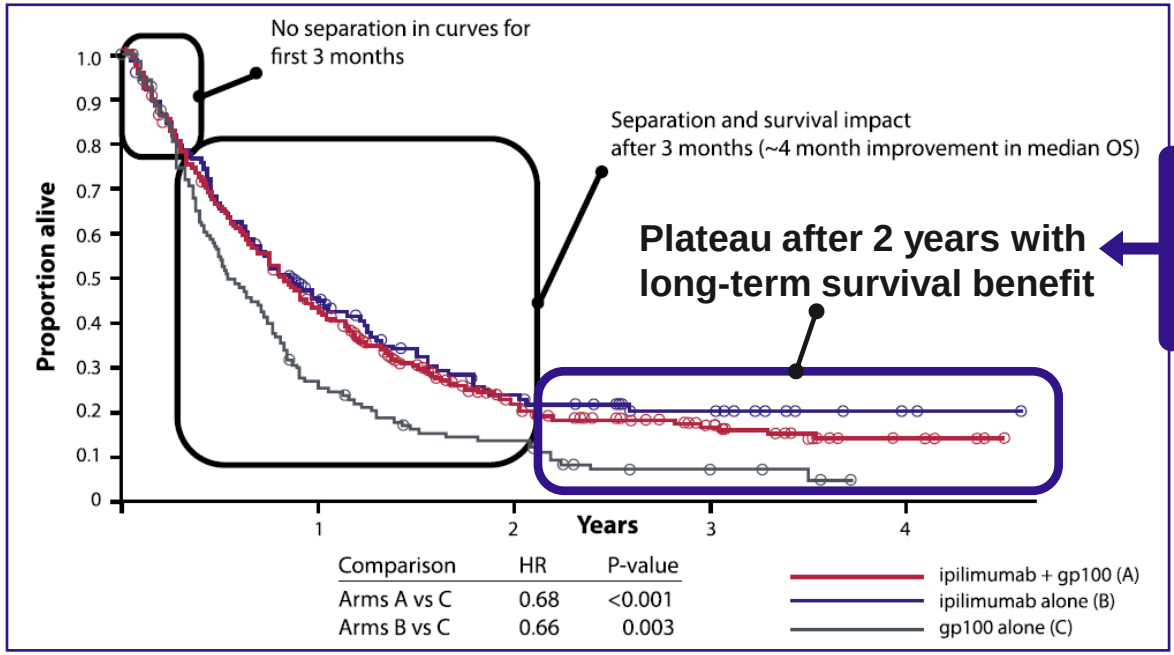
- **Tumor-infiltrating lymphocytes (TILs)**[4]
  Showed curative potential; restricted by logistics and manufacturing

1. Rosenberg SA, et al. *Nat Med*. 2004;10(9):909-915. **2.** Rosenberg SA. *J Immunol*. 2014;192(12):5451-5458. **3.** Kirkwood JM, et al. *Clin Cancer Res*. 2004;10(5):1670-1677.
4. Rosenberg SA, et al. *Nat Rev Cancer.* 2008;8(4):299-308.

The Checkpoint Inhibitor Revolution 1.0: Anti–CTLA-4

Jim Allison's notebook ("Raise the Tail")

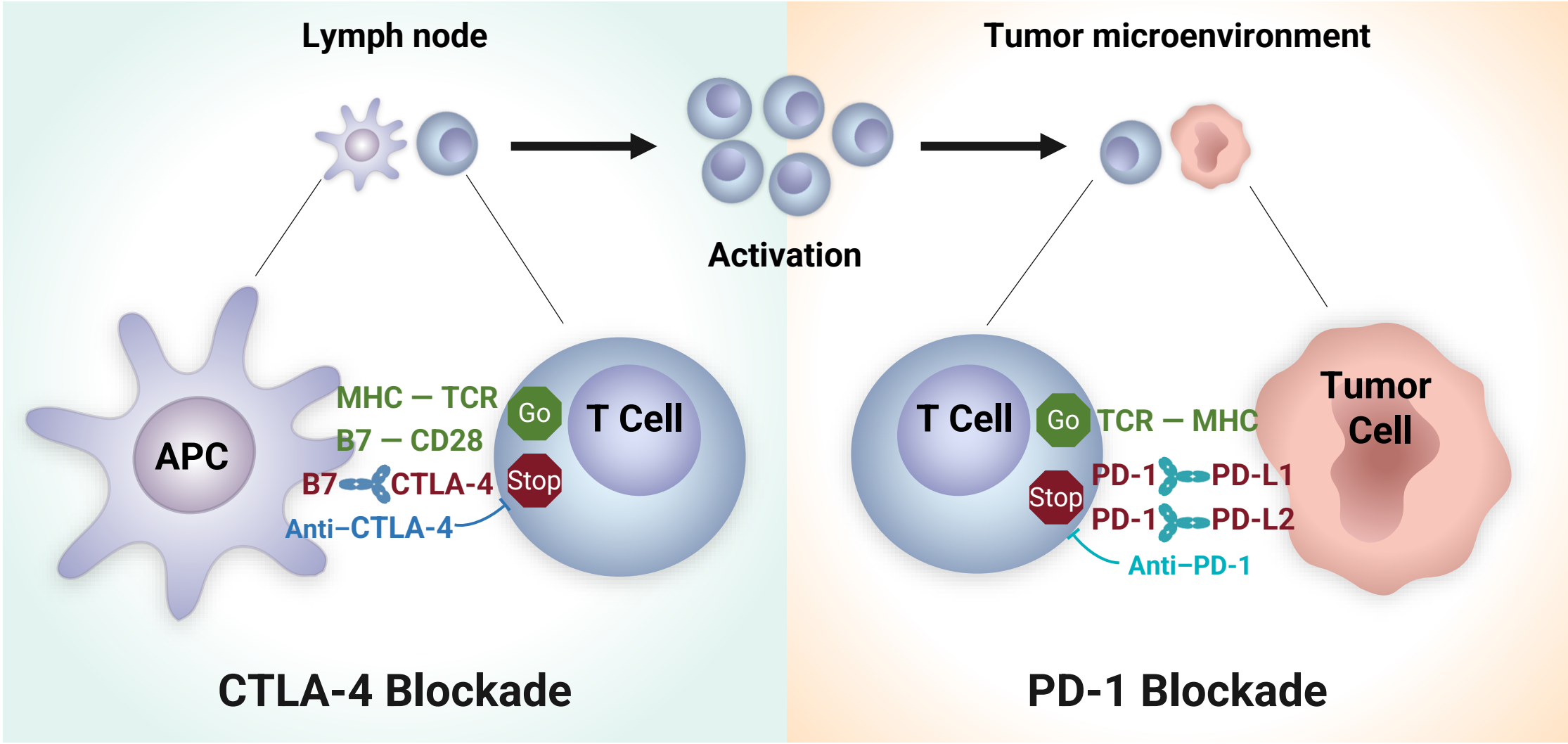




A hallmark of CTLA-4 therapy

Canavan, N. A Cure Within: Scientists Unleashing the Immune System to Kill Cancer; Hodi, O'Day et al., N Engl J Med. 2014

Blocking Distinct Pathways with CTLA-4 and PD-1



Adapted from Hassel JC, et al. *Cancer Treat Rev.* 2017;57:36-49.

Melanoma Story: Checkpoint Inhibitor Revolution 1.0

Therapies

- CTLA-4 Monotherapy
  First reproducible survival benefit[1]
- PD-1 Monotherapy
  Higer response rates, durability, & tolerability vs CTLA-4[2]
- CTLA-4/PD-1 Combination
  Highest response rates & the largest proportion of long-term survivors[3]

Key Insights Gained

- Improvement from late to early-stage disease[4]
- Neoadjuvant IO; the power of an intact primary tumor[4]
- Evolution of immune-mediated AE management[5]
- A cure for >50% of patients with widespread metastatic melanoma[3]

1. Hodi FS, et al. *N Engl J Med.* 2010;363(8):711-723. **2.** Robert C, et al. *N Engl J Med*. 2015;372(26):2521-2532. **3.** Wolchok JD, et al. *N Engl J Med.* 2025;392(1):11-22.
4. Blank CU, et al. N Engl J Med. 2024;391(18):1696-1708. **5.** Faleck DM, et al. *J Clin Oncol.* 2023;41(17):3110-3115.

Limitations of First-Generation Anti–CTLA-4

Limited monotherapy activity outside melanoma and challenging toxicity profile

- Suboptimal T-cell priming[1]
- Limited Treg depletion[2]
- Minimal activity in “cold” tumors[3]
- High-grade immune-mediated AEs[4]

1. Waight JD, et al. *Cancer Cell.* 2018;33(6):1033-1047.e5. **2.** Sharma A, et al. *Clin Cancer Res.* 2019;25(4):1233-1238. **3.** Bonaventura P, et al. *Front Immunol.* 2019;10:168.
4. Bertrand A, et al. *BMC Med.* 2015;13:211.

Developments in the Field That Led Away from CTLA-4

PD-1 replaced CTLA-4 as foundational target

PD-1 monotherapy responses and tolerability better vs CTLA-4[1]

PD-1 + non–CTLA-4 combinations

PD-1+chemo, +TKIs, +ADCs +other inhibitory pathways (i.e., TIGIT, LAG-3), etc.[2-5]

Modest success in "warm" but failure in "cold" tumors

NSCLC: PD-1+Chemo (*success*)[2]

MSS CRC: PD-1+Lenvatinib (***failure*****;** LEAP-017)[3]

NSCLC: PD-1+TIGIT (***failure*****;** SKYSCRAPER-01)[4]

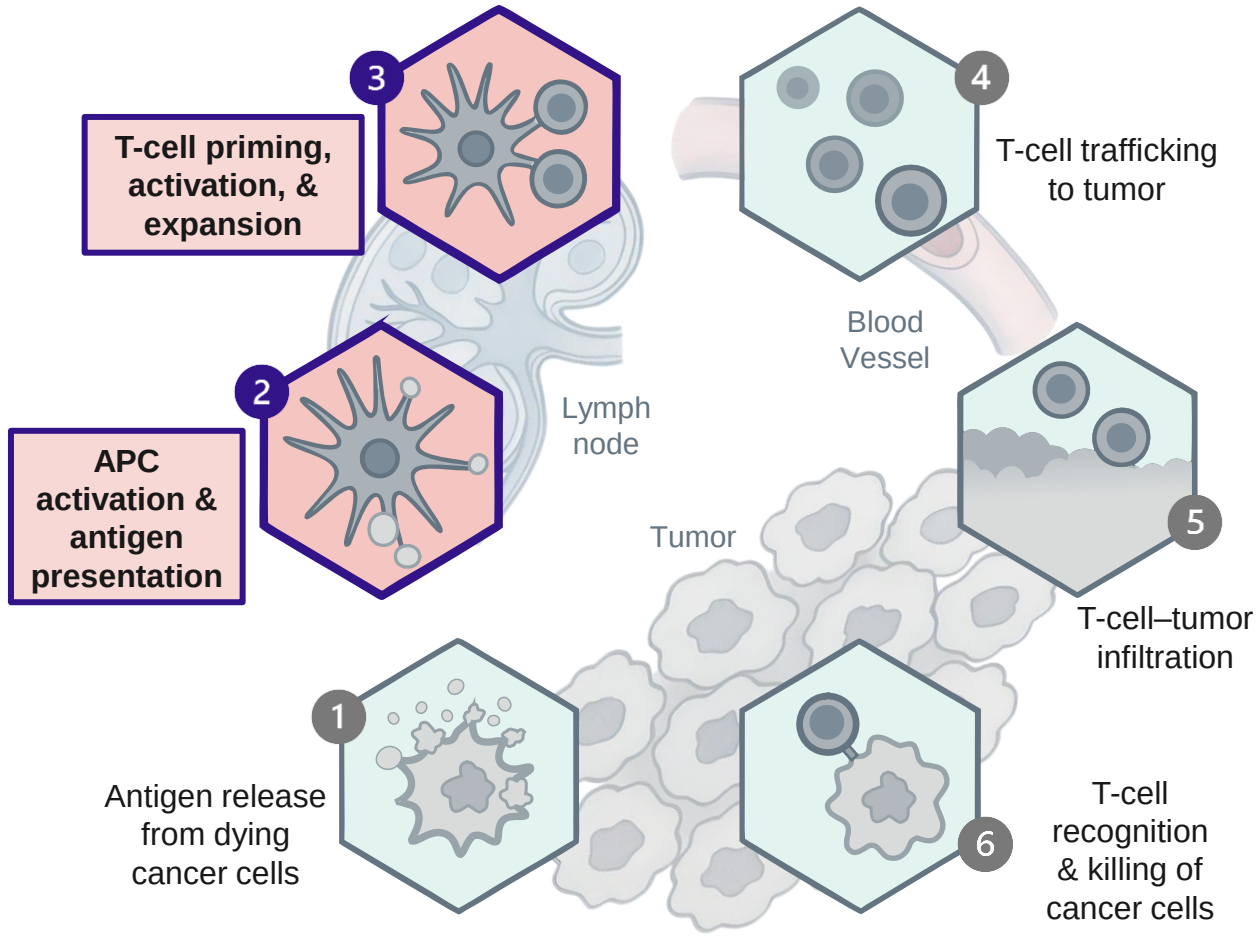
1. Robert C, et al. *N Engl J Med*. 2015;372(26):2521-2532. **2.** Pasqualotto E, et al. *Cancers (Basel).* 2023;15(21):5143. **3.** Kawazoe A, et al. *J Clin Oncol.* 2024;42(24):2918-2927.
4. Solange P, et al. *Cancer Res.* 15 April 2025; 85 (8_Supplement_2): CT051. **5.** Shi X, et al. *Front Pharmacol.* 2025;16:1556245.

IO Revolution 2.0: Priming & Expansion are Essential

Focus on targeting early activation steps in the immunity cycle

Key needs for next-gen anti–CTLA-4:

- Multifunctional immune activators
- Enhanced T-cell priming and diversity
- TME remodeling
- Improved safety
- Rational IO combinational partners beyond anti–PD-1



Adapted from Mellman I, et al. *Immunity*. 2023;56(10):2188-2205.

The Anti–CTLA-4 Challenge:
Maximizing Efficacy and Minimizing Toxicity

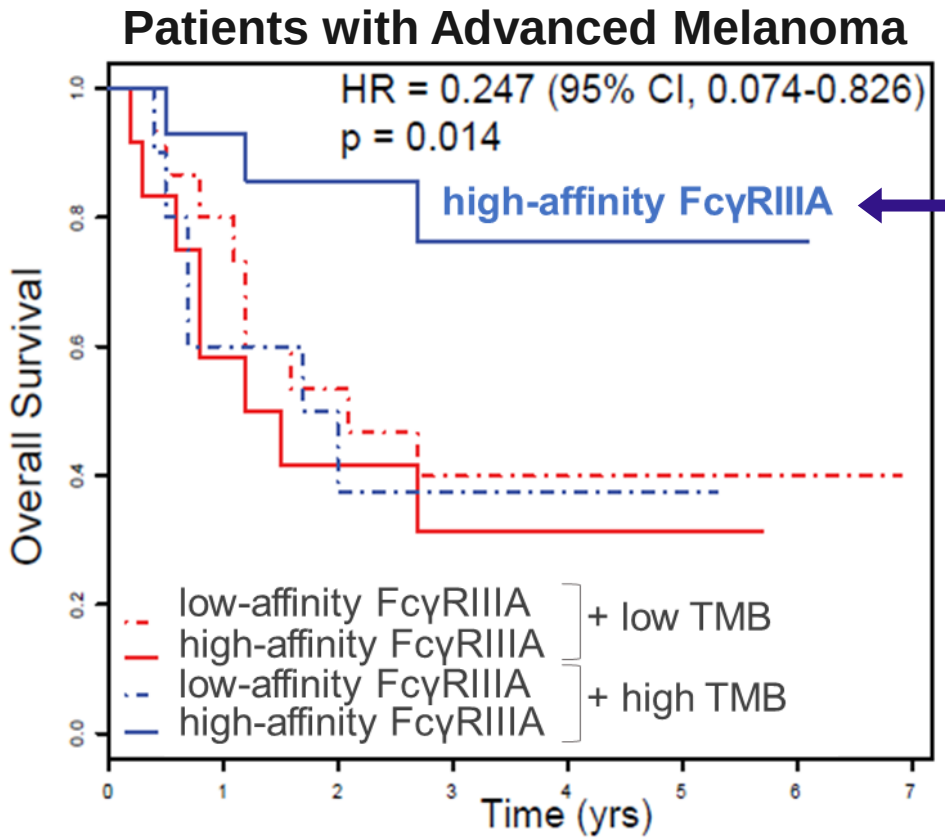
Can next-generation anti–CTLA-4 deliver more efficacy with less toxicity?

Next Generation Anti–CTLA-4

Fc-Enhanced[1,2]	Masked[3]	Bispecific[4]
Botensilimab Gotistobart Vilastobart BMS-986288	Muzastotug BMS-986249	Cadonilimab Volrustomig
Enhanced FcγR engagement improves T-cell priming, Treg depletion, & myeloid activation	**Protease-cleavable mask to localize CTLA-4 blocking activity to the TME**	**Co-engage CTLA-4 & PD-(L)1 pathways with single agent**

1. Bullock AJ, et al. N*at Med.* 2024;30(9):2558-2567 **2.** He K, et al. *J Immunother Cancer.* 2023;11(Suppl 1):A1–A1731. **3.** Bleuez C, et al. *Drug Discov Today*. 2022;27(6):1743-1754.
4. Shan KS, et al. *Int J Mol Sci.* 2025;26(12):5838.

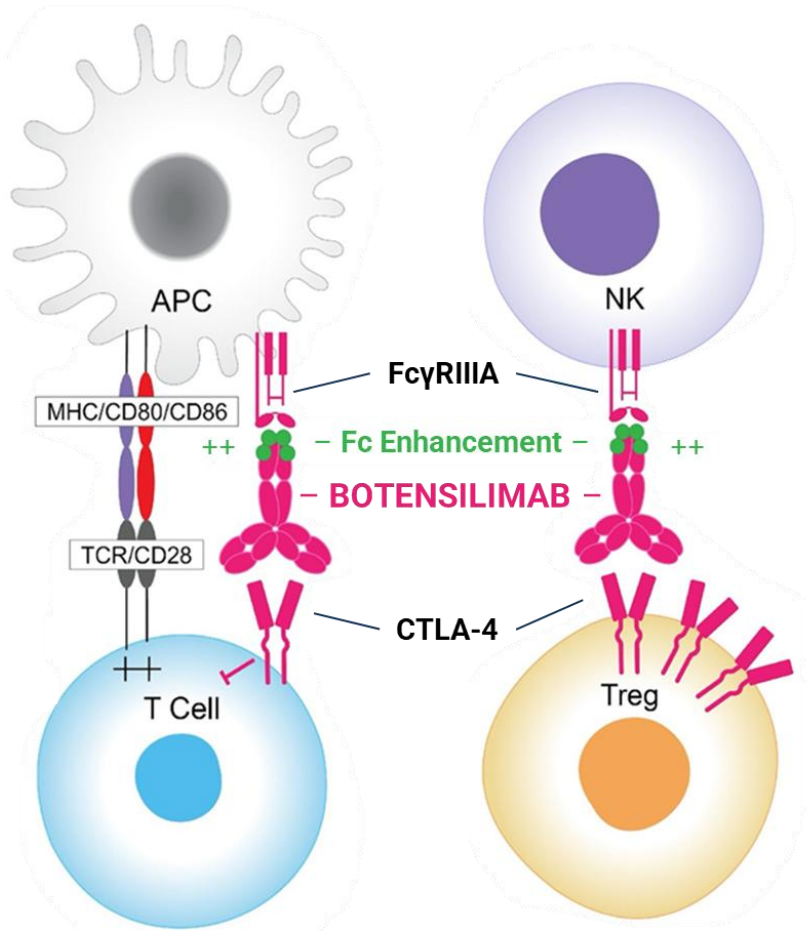
Importance of Fc-FcγR Interactions: Evidence from Ipilimumab



Better clinical outcomes in patients with high-affinity FcγRIIIA polymorphism

Adapted from Vargas F, et al. *Cancer Cell.* 2018;33(4):649-663. (Licensed under CC-BY 4.0).

Botensilimab: A Multifunctional Immune Activator



Active in cold and IO-refractory tumors

Botensilimab (BOT)[1-3]

Multifunctional, Fc-enhanced CTLA-4 Inhibitor

1) **Enhances T-Cell Priming, Activation and Memory**
Primes and expands a diverse set of tumor-reactive T cells that can infiltrate the tumor; establishes memory

2) **Activates APCs/Myeloid Cells**
Upregulates co-stimulatory and antigen presentation machinery on dendritic cells and other myeloid cells

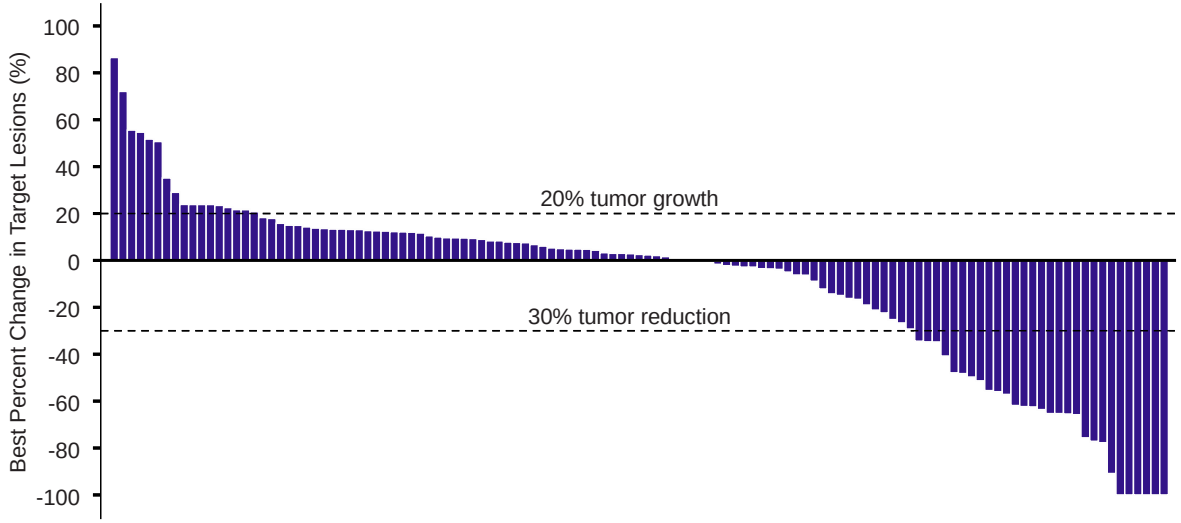
3) **Reduces Regulatory T Cells**
Removes intratumoral regulatory T cells that suppress the activity of cytotoxic T cells

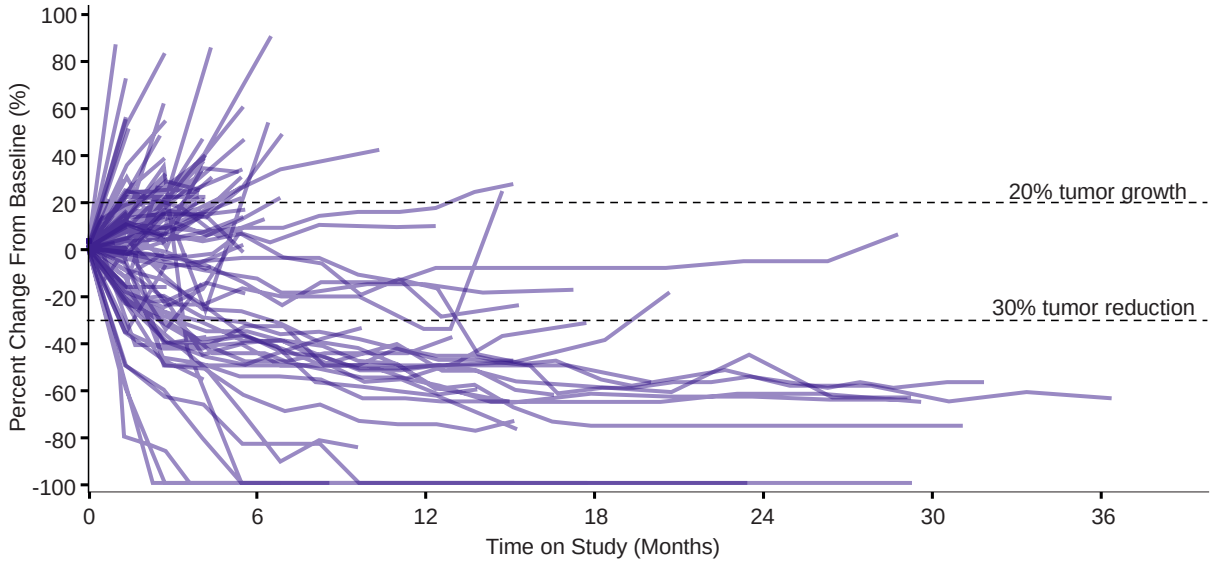
4) **Avoids Difficult-To-Treat Immune-Related AEs**
Introduction of a point mutation mitigates complement-mediated toxicities associated with conventional anti-CTLA-4 therapy

1. Bullock AJ, et al. *Nat Med.* 2024;30(9):2558-2567. **2.** Waight JD, et al. *Cancer Cell.* 2018;33(6):1033-1047.e5. **3.** Chand D, et al. *Cancer Discov.* 2024;14(12):2407-2429.

“Cold” 3L+ MSS mCRC NLM: Best Overall Responses (N=123)

Phase 1b C-800-01 trial





Objective Response Rate
20%
95% CI, 13–28

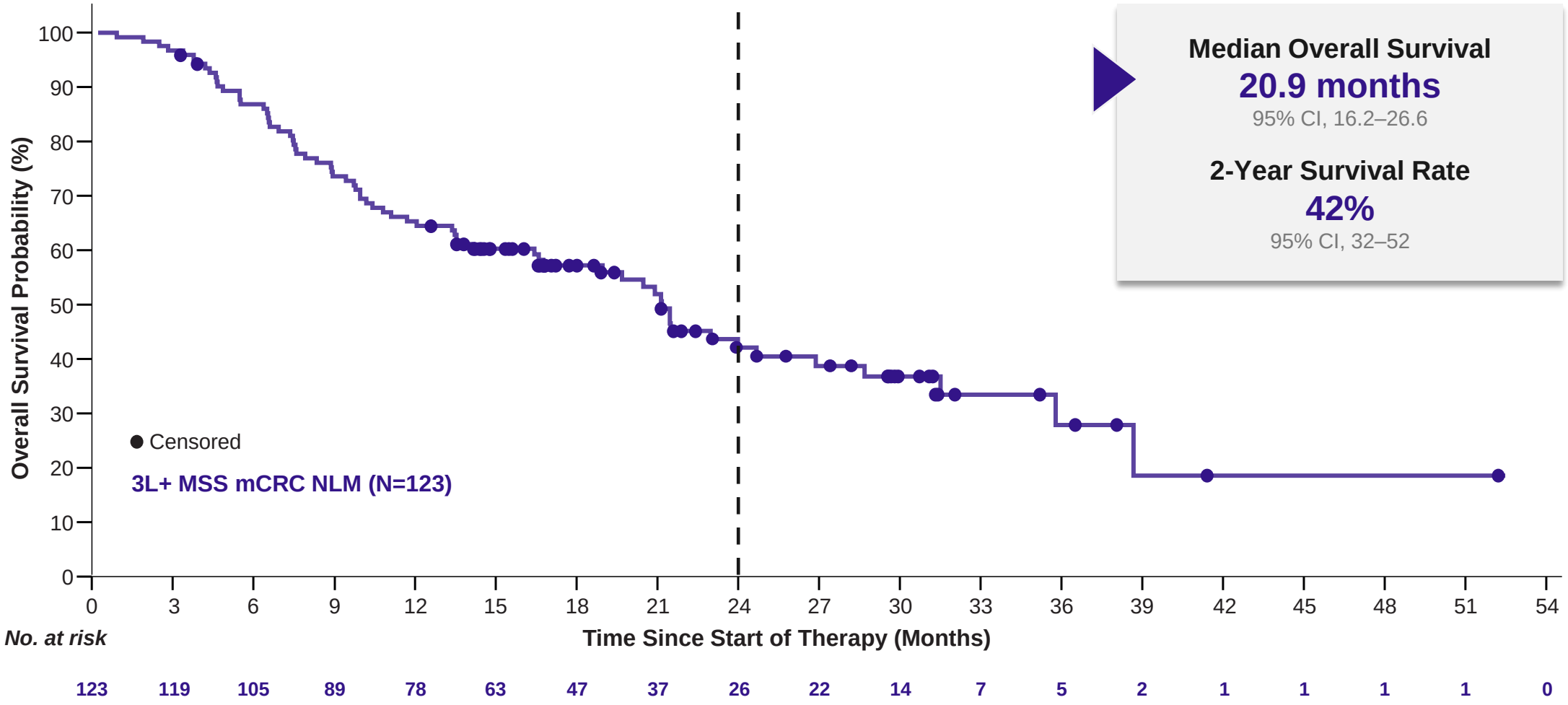
Median Duration of Response
16.6 months
95% CI, 5.7–NR

Disease Control Rate
69%
95% CI, 60–77

Schlechter BM, et al. Poster presented at the ESMO GI Congress. Barcelona, Spain. 2025. Poster #8P. Data cutoff: 13-Mar-2025.

“Cold” 3L+ MSS mCRC NLM: Overall Survival

Phase 1b C-800-01 trial



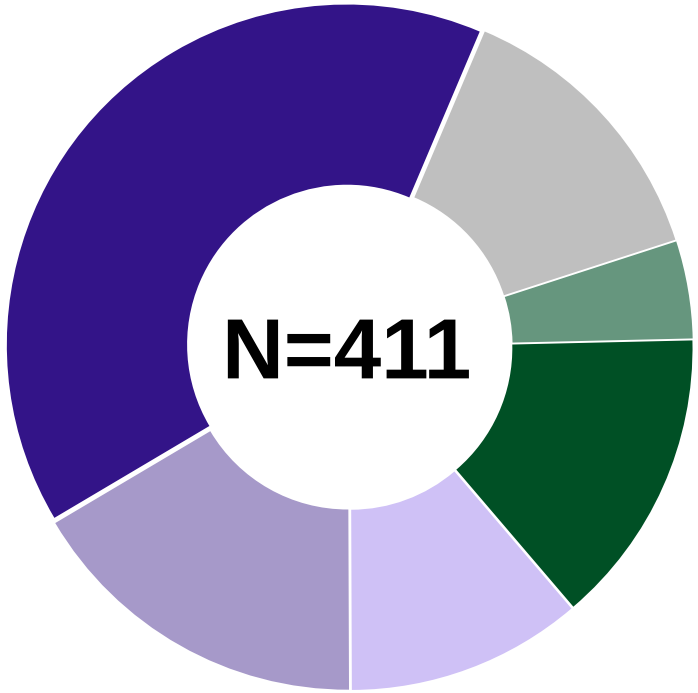
Median Overall Survival
20.9 months
95% CI, 16.2–26.6

2-Year Survival Rate
42%
95% CI, 32–52

Schlechter BM, et al. Poster presented at the ESMO GI Congress. Barcelona, Spain. 2025. Poster #8P. Data cutoff: 13-Mar-2025.

Pan-Tumor Study of BOT/BAL: “Cold” and IO R/R Cancers

Phase 1b C-800-01 trial



Cold Tumors

- MSS mCRC (n=164)
- Sarcoma (n=68)
- Ovarian (n=46)

I-O R/R

- PD-1 R/R NSCLC (n=58)
- PD-(L)1 R/R HCC (n=19)
- Other (n=56)
  Endometrial (n=14), pancreatic (n=14), prostate (n=4), melanoma (n=3) and other[a]

61% **Received ≥3 prior lines of treatment**

33% **Received prior PD-(L)1 and/or CTLA-4 treatment**

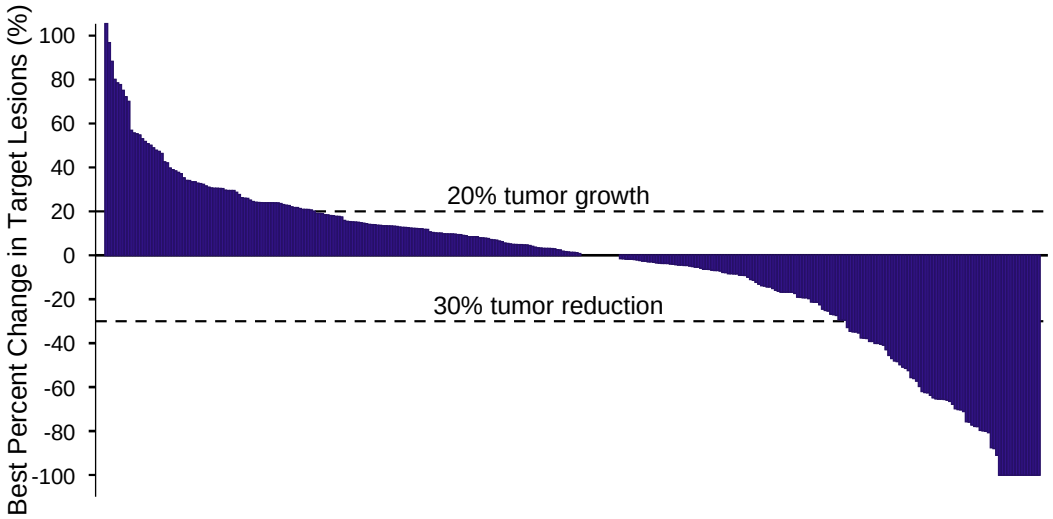
Data cutoff: 13-Mar-2025. ClinicalTrials.gov Identifier: NCT03860272.

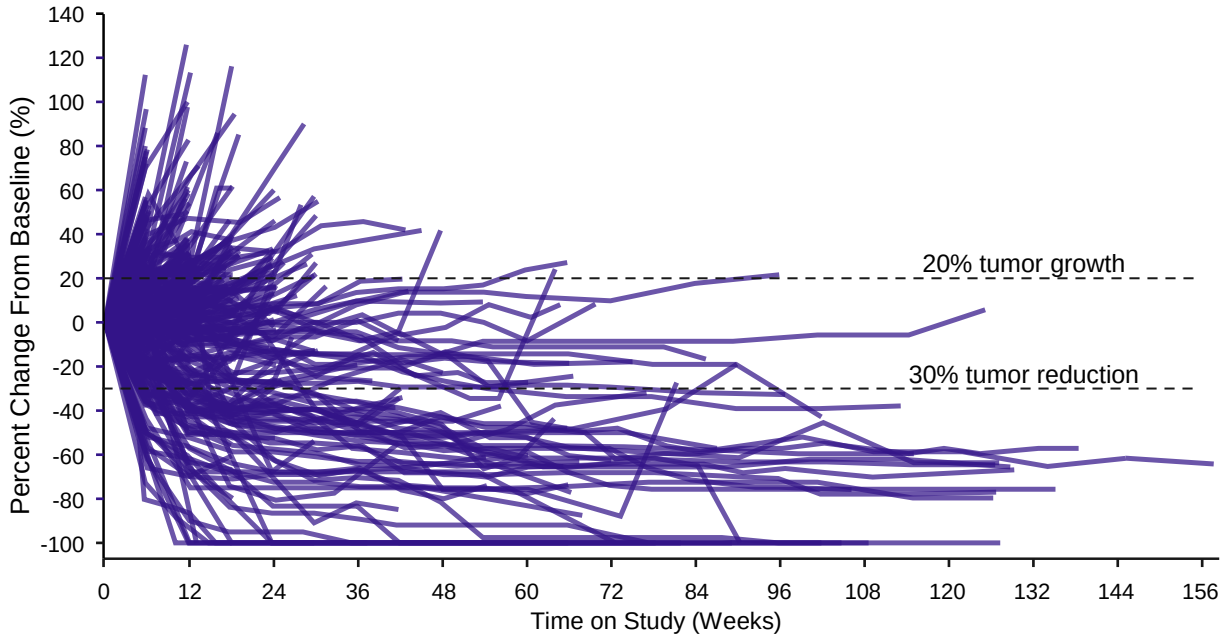
[a]Less than 2 patients per indication: Adrenocortical carcinoma, ampullary, anal cancer, breast cancer, carcinoma of unknown primary, cervical cancer, digital papillary, esophageal cancer, fibrolamellar hepatocellular cancer, gallbladder cancer, gastric cancer, head and neck cancer, neuroendocrine carcinoma, renal cell carcinoma, testicular cancer, urothelial carcinoma.

Gordon MS, et al. Oral Presentation at ESMO Annual Meeting. Berlin, Germany. 2025. #1517MO. Data cutoff: 13-MAR-2025.

9+ Tumor Types: Best Overall Responses (n=339)

Phase 1b C-800-01 trial





Objective Response Rate
17%
95% CI, 13–22

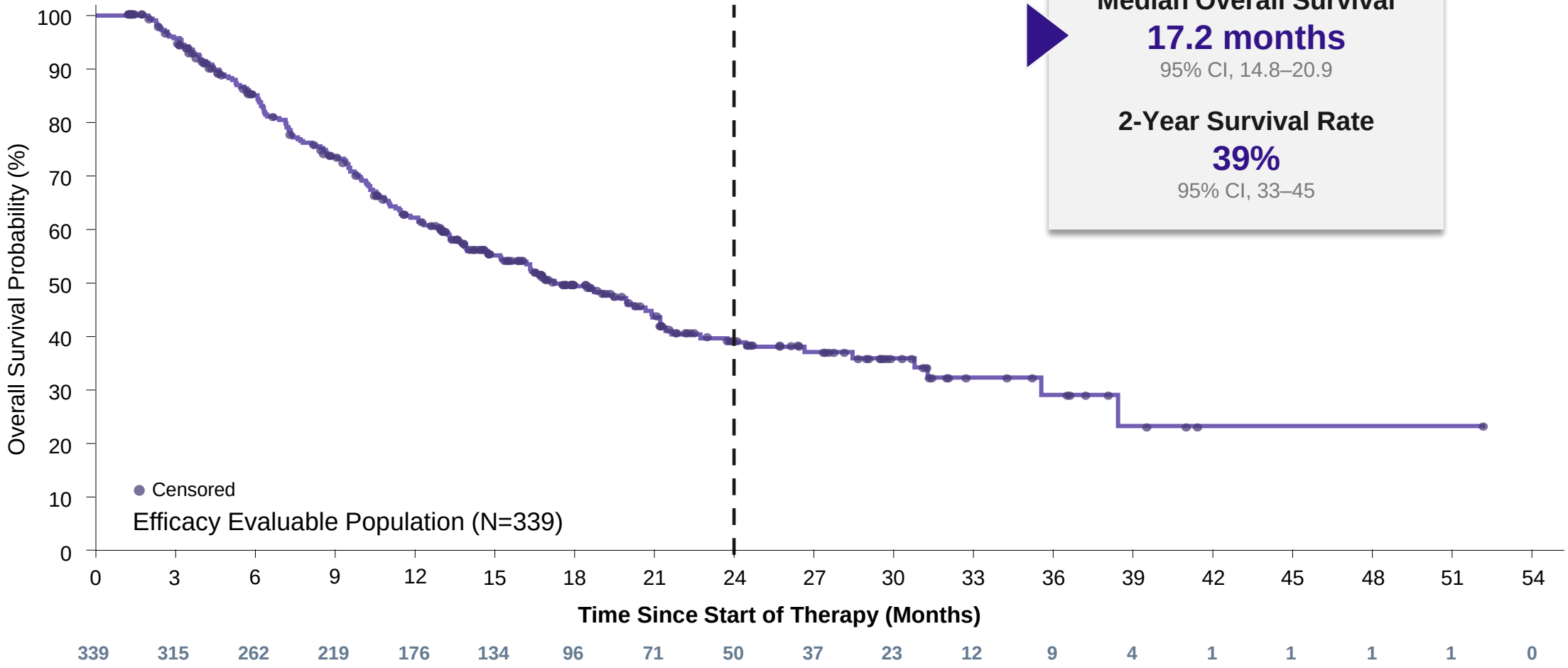
Median Duration of Response
14.6 months
95% CI, 9.7–NR

Disease Control Rate
66%
95% CI, 60–71

Per investigator assessment in efficacy evaluable set (N=339; participants who received ≥1 post-baseline 6-week imaging scan).
Gordon MS, et al. Oral Presentation at ESMO Annual Meeting. Berlin, Germany. 2025. #1517MO. Data cutoff: 13-MAR-2025.

9+ Tumor Types: Overall Survival

Phase 1b C-800-01 trial



Median Overall Survival
17.2 months
95% CI, 14.8–20.9

2-Year Survival Rate
39%
95% CI, 33–45

Per investigator assessment in efficacy evaluable set (N=339; participants who received ≥1 post-baseline 6-week imaging scan).
Gordon MS, et al. Oral Presentation at ESMO Annual Meeting. Berlin, Germany. 2025. #1517MO. Data cutoff: 13-MAR-2025.

9+ Tumor Types: Safety Overview

Phase 1b C-800-01 trial

Safety event, n (%)	1 mg/kg (n=228)		2 mg/kg (n=183)		Overall (N=411)	
Any grade TRAE	**194 (85)**		**157 (86)**		**351 (85)**	
Grade ≥3 TRAEs	63 (28)		69 (38)		132 (32)	
	Any grade	**Grade ≥3**	**Any grade**	**Grade ≥3**	**Any grade**	**Grade ≥3**
Any treatment-related imAE[a,b]	96 (42)	42 (18)	99 (54)	49 (27)	195 (47)	91 (22)
Most common (≥3%) treatment-related imAEs[a]						
Diarrhea/colitis[c]	61 (27)	22 **(10)**	73 (40)	34 (19)	134 (33)	56 (14)
Thyroid[d]	17 (8)	**0**	15 (8)	0	32 (8)	0
Hepatitis[e]	6 (3)	2 **(1)**	13 (7)	8 (4)	19 (5)	10 (2)
Skin[f]	4 (2)	2 **(1)**	9 (5)	3 (2)	13 (3)	5 (1)
Pneumonitis[g]	5 (2)	3 **(1)**	6 (3)	2 (1)	11 (3)	5 (1)

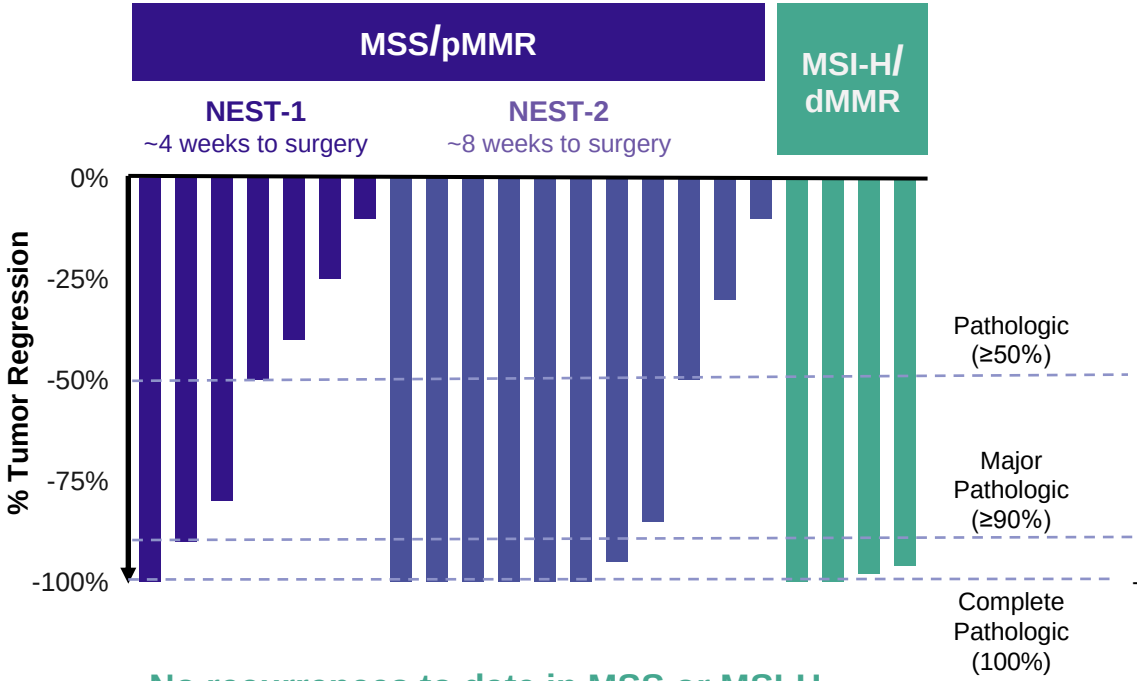
- The most common imAEs were GI-related, which were reversible
- Low incidence of visceral toxicities outside the GI tract
- No treatment-related deaths were observed (grade 5)

Safety analysis set (N=411; participants who received ≥1 dose of study drug). Gordon MS, et al. Oral Presentation at ESMO Annual Meeting. Berlin, Germany. 2025. #1517MO. Data cutoff: 13-MAR-2025.

[a]imAEs were medically adjudicated. [b]Grade 4 imAEs (n=1 each) of colitis (2 mg/kg group), autonomic neuropathy (1 mg/kg group), diabetic ketoacidosis (2 mg/kg group), and thrombocytopenia (1 mg/kg group) were reported; no other grade ≥4 imAEs occurred. [c]Grouped term that included preferred term events of autoimmune colitis, colitis, diarrhea, duodenitis, enteritis, enterocolitis, and immune-mediated enterocolitis. [d]Grouped term that included preferred term events of blood thyroid stimulating hormone increased, hyperthyroidism, hypothyroidism, immune-mediated hypothyroidism, immune-mediated thyroiditis, and thyroiditis. [e]Grouped term that included preferred term events of AST increased, ALT increased, autoimmune hepatitis, blood alkaline phosphatase increased, and immune-mediated hepatitis. [f]Grouped term that included preferred term events of immune-mediated dermatitis, lichen sclerosus, linear IgA disease, rash, rash erythematous, and rash maculo-papular that were treated systemically. [g]Grouped term that included preferred term events of immune-mediated lung disease, and pneumonitis.

Neoadjuvant CRC: Responses

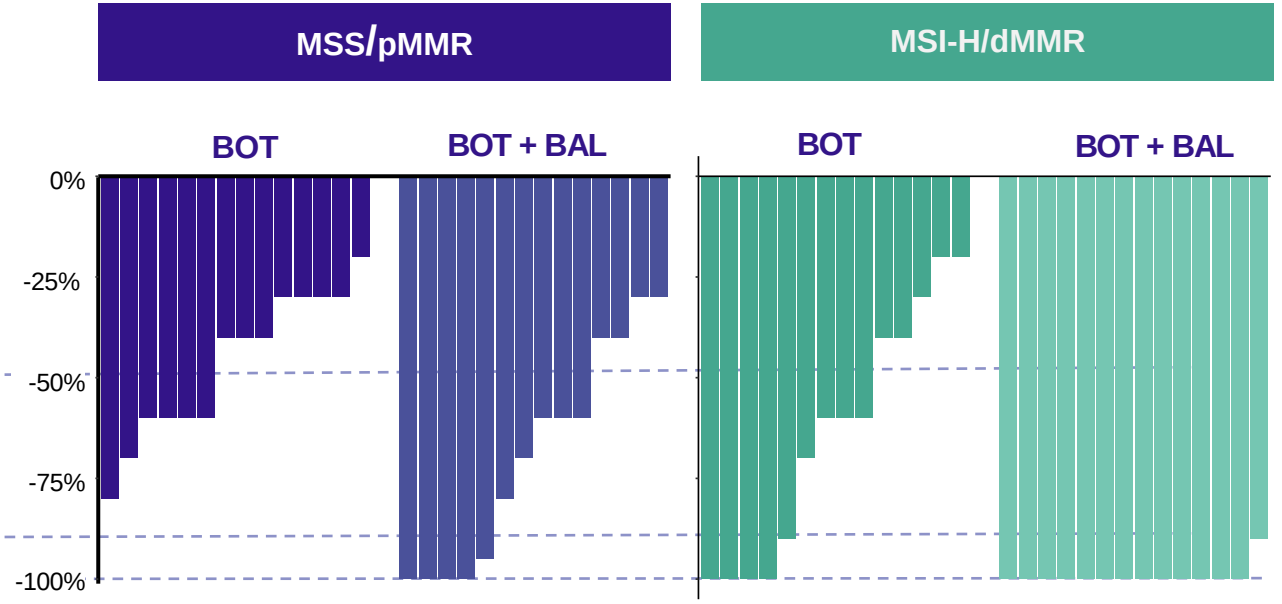
NEST (BOT+BAL)[1]



No recurrences to date in MSS or MSI-H

Median follow-up:
NEST-1: 18.2 months (IQR 16.9-19.2)
NEST-2: 8.98 months (IQR 8.1-9.4)

UNICORN (BOT±BAL)[2]



No recurrences with BOT + BAL (median follow-up 6–9 months)

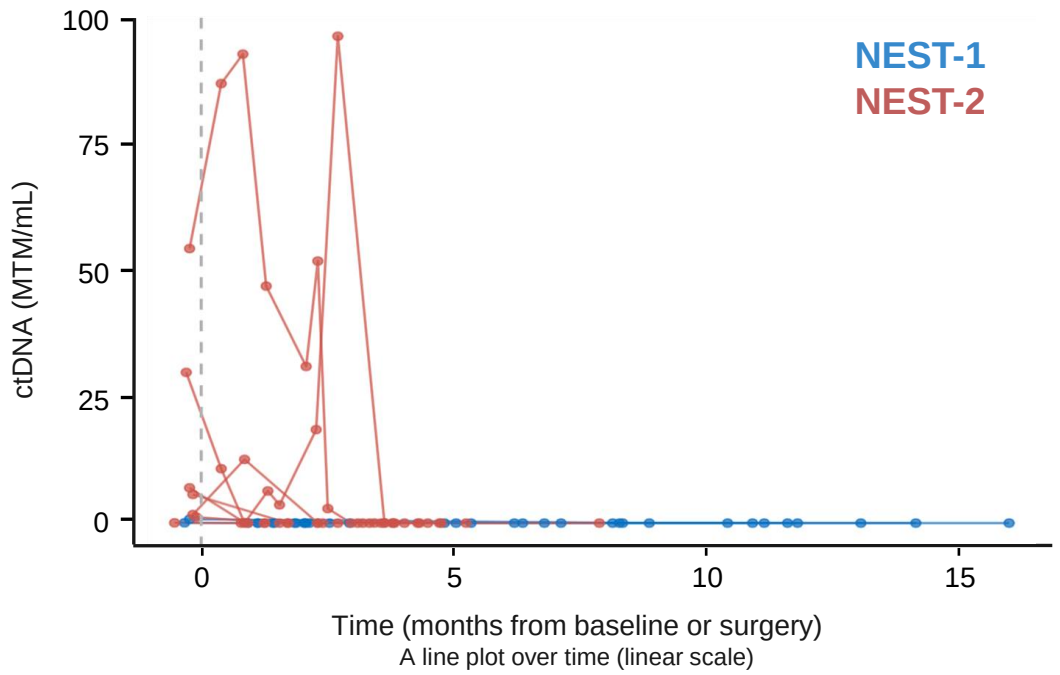
4 patients progressed with BOT monotherapy (median follow-up 11–13 months); these patients did not have initial pathologic response

1. Hissong E, et al. Poster presented at the 2025 ASCO GI Congress. Chicago, IL, USA. Abstract #207. Phase 2 ClinicalTrials.gov Identifier: NCT05571293.
2. Ghelardi F, et al. Poster presented at the 2025 ASCO GI Congress. Chicago, IL, USA. Poster #F20. Phase 2 ClinicalTrials.gov Identifier: NCT05845450

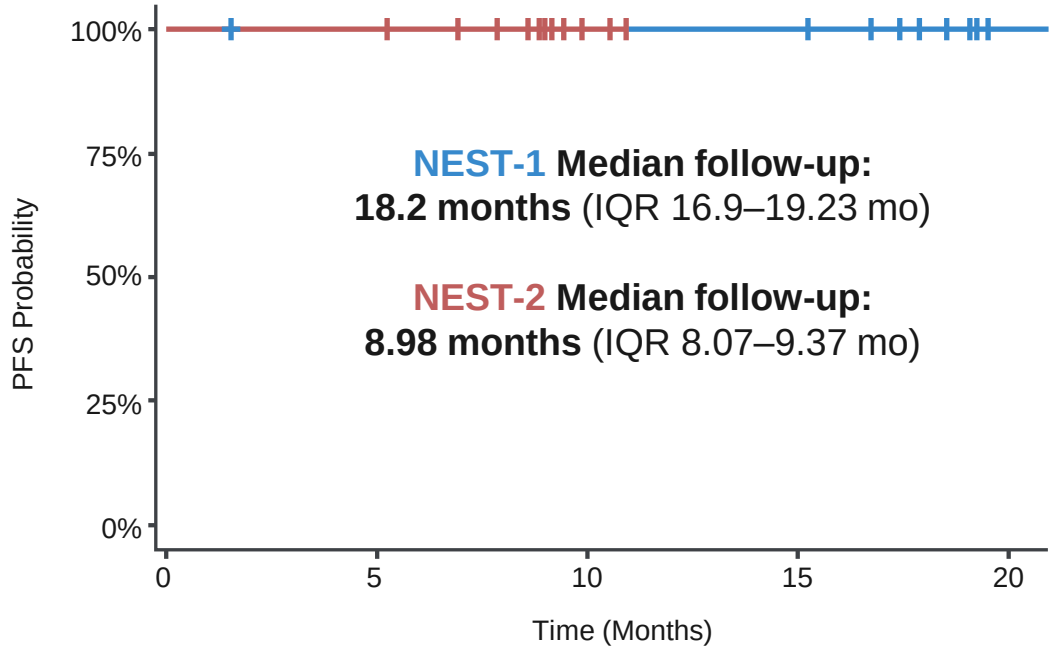
Neoadjuvant CRC: ctDNA and PFS

NEST (BOT+BAL)

ctDNA Levels Over Time



Progression-Free Survival

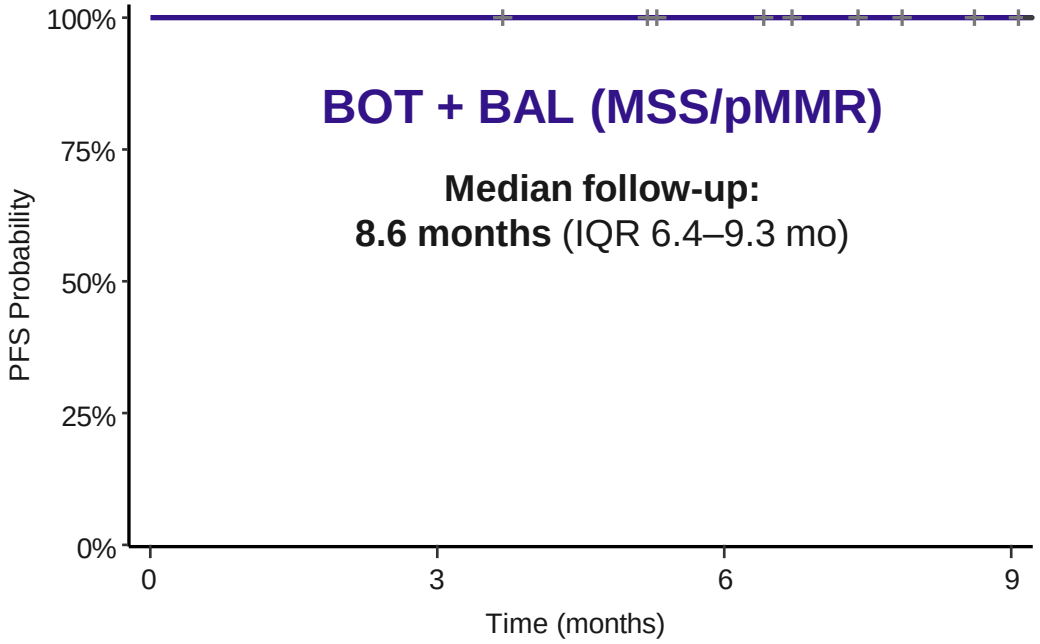


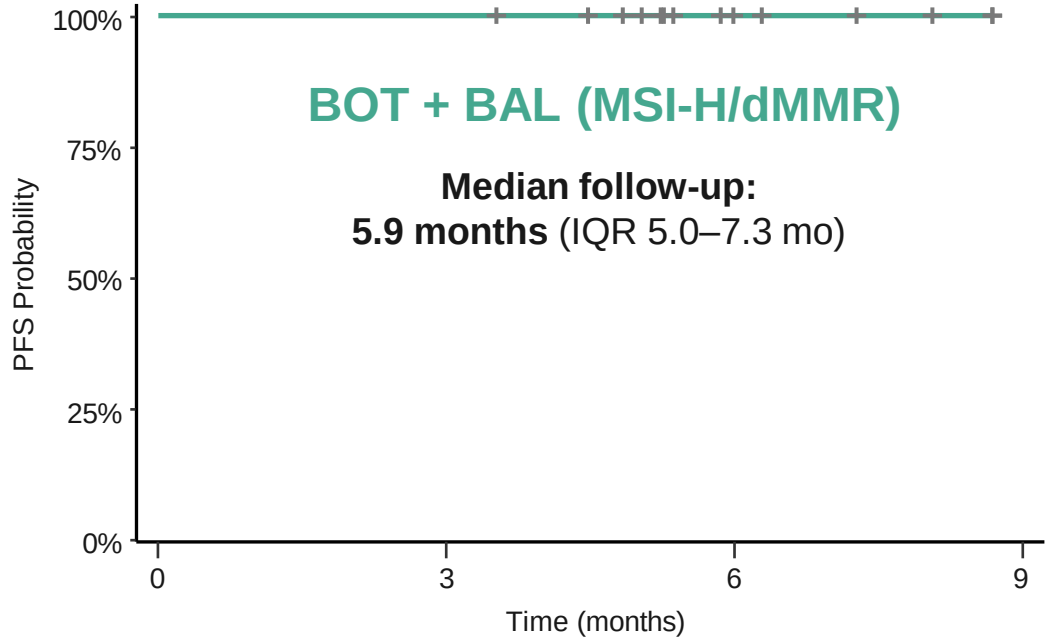
Hissong E, et al. Poster presented at the 2025 ASCO GI Congress. Chicago, IL, USA. Abstract #207. Phase 2 ClinicalTrials.gov Identifier: NCT05571293.

Neoadjuvant CRC: PFS

UNICORN (BOT±BAL)[2]

Progression-Free Survival





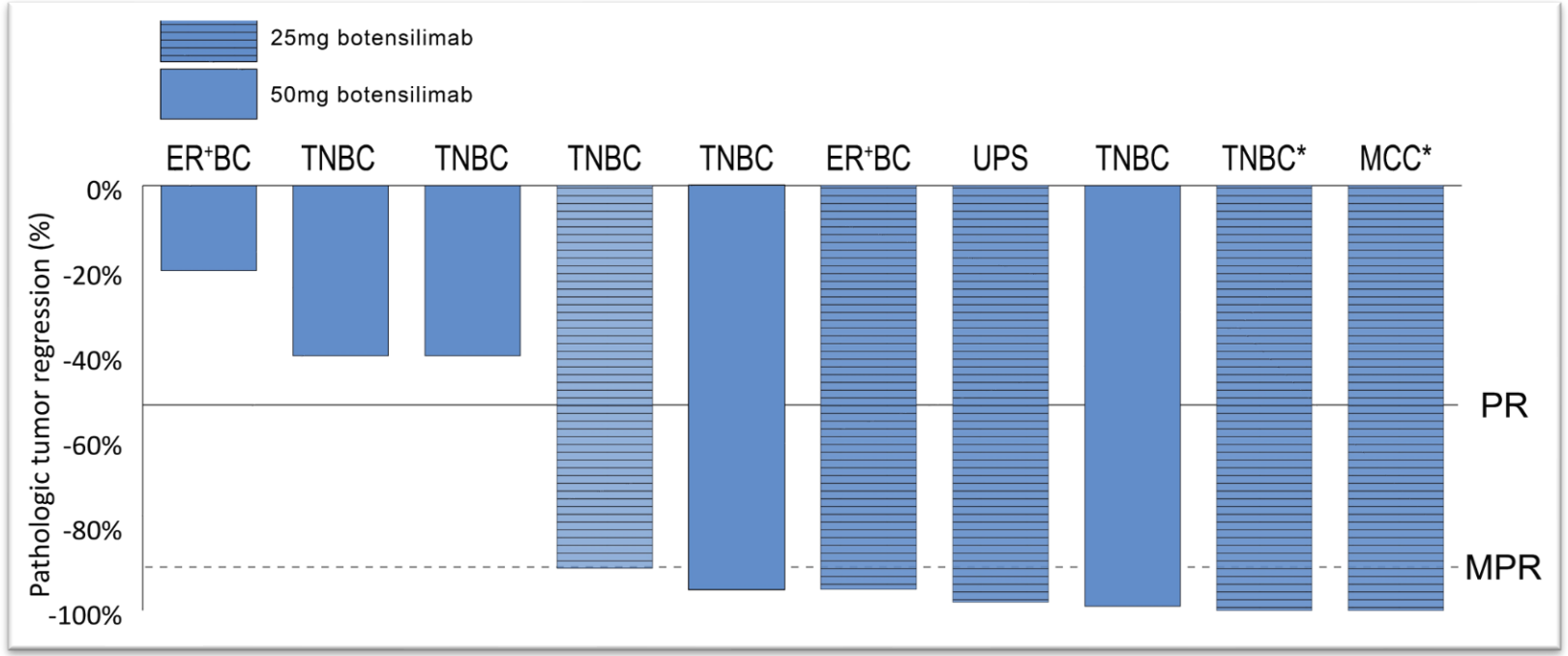
Ghelardi F, et al. Poster presented at the 2025 ASCO GI Congress. Chicago, IL, USA. Poster #F20. Phase 2 ClinicalTrials.gov Identifier: NCT05845450

BOT+BAL Neoadjuvant Across Tumor Types

NEOASIS (BOT+BAL)

MSS/pMMR

~8 weeks to surgery



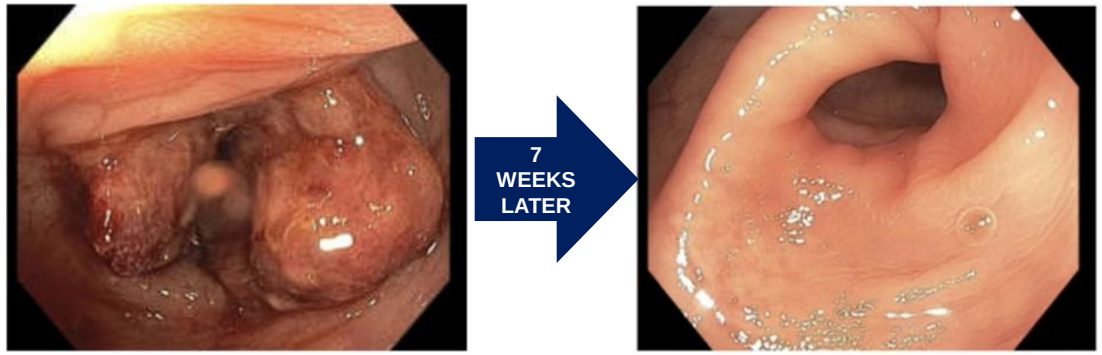
70% major pathologic response rate overall (with both BOT doses)

ER+BC, estrogen receptor positive breast; MCC, Merkel cell carcinoma; MPR, major pathologic response; pMMR, mismatch repair proficient; PR, pathologic response; TNBC, triple negative breast cancer; UPS, undifferentiated pleiomorf sarcoma.

Chalabi M, et al. Oral presentation as AACR 2025. Chicago, IL, USA. Abstract #CT130. Phase 2 ClinicalTrials.gov Identifier: NCT06279130.

Potentiating Antitumor Immunity with BOT: Fc-Enhanced anti–CTLA-4

In MSS CRC[1]



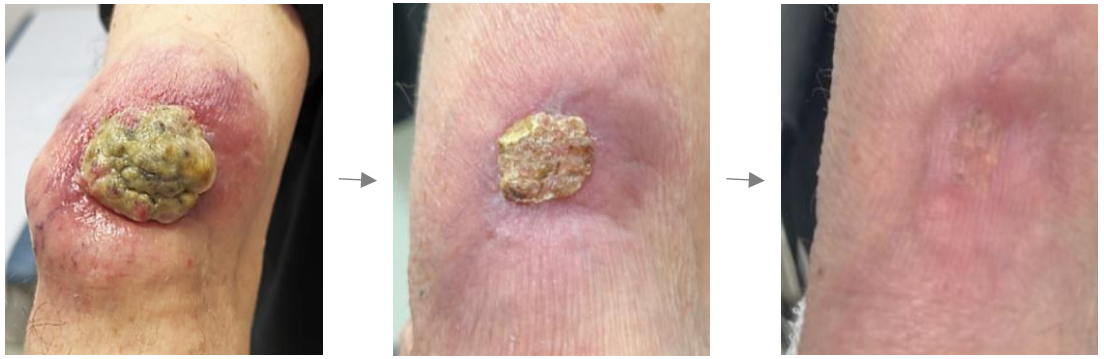
Pre-BOT/BAL Treatment | **Post-BOT/BAL Treatment**

Stage III MSS Colon Cancer (8 cm tumor)

1 dose of BOT + 2 doses of BAL during 7-week period pre-surgery

Patient received no prior nor concurrent treatments

And Other Solid Tumors[2]



Baseline | **3 Weeks Post BOT/BAL** | **6 Weeks Post BOT/BAL**

Merkel cell carcinoma, left elbow

Clear macroscopic regression after 3 weeks

100% regression including multiple lymph nodes

1. Kasi P, et al. Oral presentation at the ESMO Gastrointestinal Cancers Congress. Munich, Germany. 2024. Presentation #743.
2. Chalabi M, et al. Oral presentation at AACR. Chicago, IL, USA. 2025. Abstract #CT130.

The IO Revolution 2.0 Has Begun

Insights Gained from IO Revolution 1.0:

- First-generation anti–CTLA-4 therapies launched the IO Revolution 1.0 in melanoma[1-3]
  - Delivered unprecedented durable remissions and cures in patients with metastatic disease following a short treatment course
- First-generation CTLA-4 ± PD-1 demonstrated minimal activity in “cold” tumors and high-grade immune-mediated AEs[4-5]
- Improved T-cell priming/activation, memory, Treg depletion, and TME remodeling are key features needed to broaden IO success in poorly immunogenic tumors[6]

1. Hodi FS, et al. *N Engl J Med.* 2010;363(8):711-723. **2.** Robert C, et al. *N Engl J Med*. 2015;372(26):2521-2532. **3.** Wolchok JD, et al. *N Engl J Med.* 2025;392(1):11-22. **4.** Bonaventura P, et al. *Front Immunol.* 2019;10:168. **5.** Bertrand A, et al. *BMC Med.* 2015;13:211. **6.** Waight JD, et al. *Cancer Cell.* 2018;33(6):1033-1047.e5.

The IO Revolution 2.0 Has Begun (cont'd)

Moving Forward Towards IO Revolution 2.0:

- Multiple next-generation anti–CTLA-4 antibodies have been designed to broaden efficacy and improve safety; these are rapidly advancing in the clinic and providing strong proof of concept[1]
- Anti–CTLA-4 Fc-enhancement is a critical feature of next-generation agents, driving improved T-cell priming, Treg depletion, and TME remodeling[2]
- Next-generation anti–CTLA-4 will serve as foundational agents in the IO Revolution 2.0 by:
  - Making poorly immunogenic tumors visible to the immune system[2]
  - Allowing multiple combination partners to help drive tumor eradication and limit/prevent resistance[1]

1. Rudqvist NP, et al. *Oncoimmunology.* 2023;12(1):2275333. **2.** Waight JD, et al. *Cancer Cell.* 2018;33(6):1033-1047.e5.

Acknowledgements

I would like to thank the congress organizers for the opportunity to speak, and to acknowledge the patients, families, and clinical teams whose contributions and participation made the presented studies possible.

Abbreviations

ADC, antibody-drug conjugate
AE, adverse event
ALT, alanine aminotransferase
APC, antigen-presenting cell
AST, aspartate aminotransferase
BAL, balstilimab
BOT, botensilimab
Chemo, chemotherapy
CI, confidence interval
CRC, colorectal cancer
ctDNA, circulating tumor DNA
CTLA-4, cytotoxic T-lymphocyte-associated protein-4
DCR, disease control rate (complete or partial response, or stable disease ≥6 weeks)
dMMR, deficient mismatch repair
Fc, fragment crystallizable
FcγRIIIA, Fc gamma receptor III-A
GI, gastrointestinal
Gp100, glycoprotein 100
HCC, hepatocellular carcinoma
HR, hazard ratio
IFN-α, interferon alpha
IgA, immunoglobulin A
IL-2, interleukin 2
imAE, immune-mediated adverse event
IO, immuno-oncology
IQR, interquartile range
LAG-3, lymphocyte-activation gene 3
mCRC, metastatic colorectal cancer
MHC, major histocompatibility complex
MSI-H, microsatellite instability-high
MSS, microsatellite stable
MTM, mean tumor molecules
NK, natural killer
NLM, no liver metastases
NR, not reached
NSCLC, non-small cell lung cancer
OS, overall survival
PD-1, programmed cell death protein 1
PD-L1, programmed death ligand 1
PFS, progression-free survival
pMMR, proficient mismatch repair
RCC, renal cell carcinoma
R/R, relapsed/refractory
TCR, T-cell receptor
TIGIT, T cell immunoreceptor with Ig & ITIM domains
TKIs, tyrosine kinase inhibitors
TMB, tumor mutational burden
TME, tumor microenvironment
TRAE, treatment-related adverse event
Treg, T-regulatory cell
yrs, years

Steven J. O'Day, MD