MELANOMA

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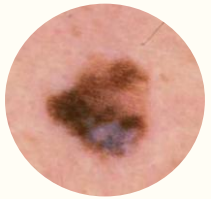
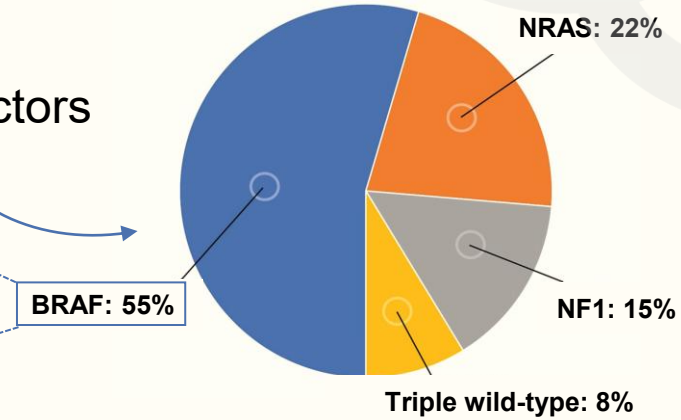
BACKGROUND: CUTANEOUS MELANOMA



Cutanous Melanoma (**CM**) is a life-threathening tumor arising from melanocytes

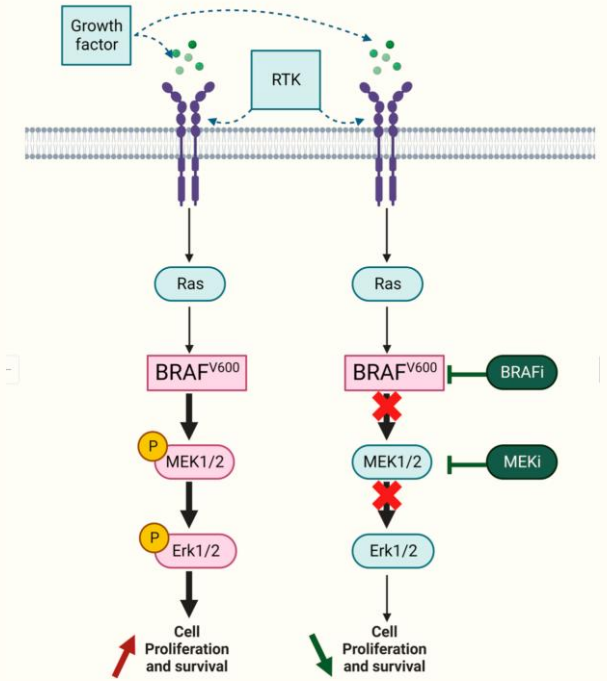
- Increasing incidence worldwide: 324,635 new cases/year
- Complex pathogenesis: phenotypic, environmental ang **genetic** factors

BRAF**V600E/K/R** mutations drive multiple **oncogenic mechanisms**



FDA

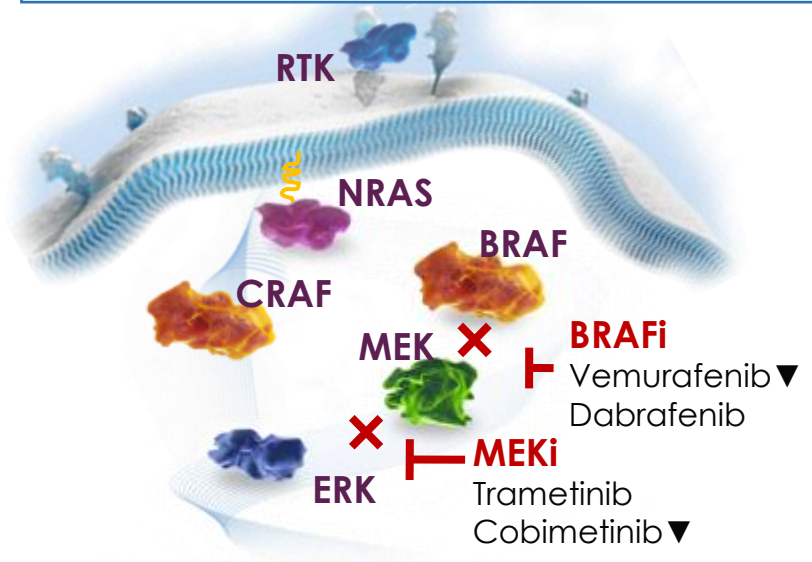
- BRAF-mutated patients: targeted therapy (**TT**) BRAF plus MEK inhibitors
- BRAF-WT patients*:* immune checkpoint inhibitors (**ICI**) (e.g. anti-PD-1, CTLA-4)



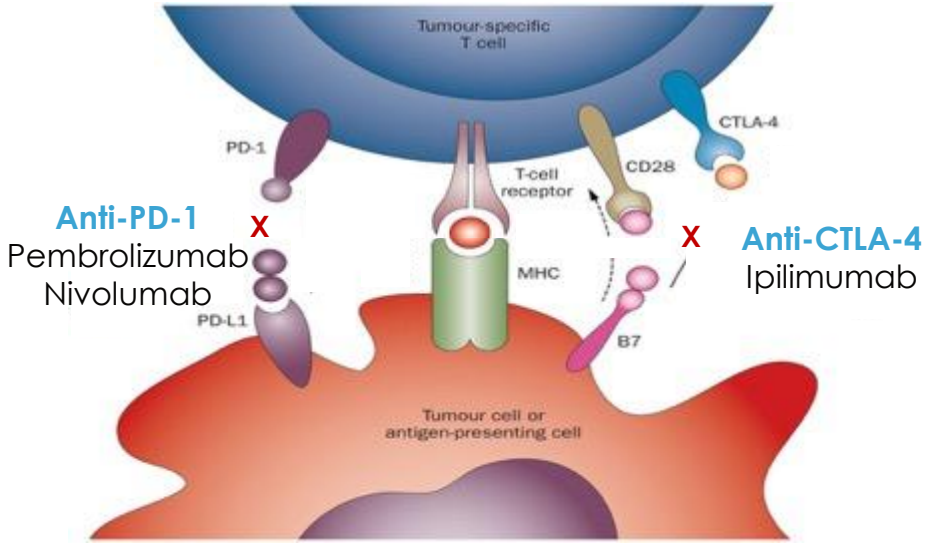
Images adapted from Florent L., Cancers, 2023 ; Ortiz-Urda S., et al, Melanoma, 2024

Advances in our understanding of the biology of melanoma have led to the development of new classes of therapy

Targeted therapies
Interfere with regulatory molecules involved in cancer cell growth and proliferation[1]



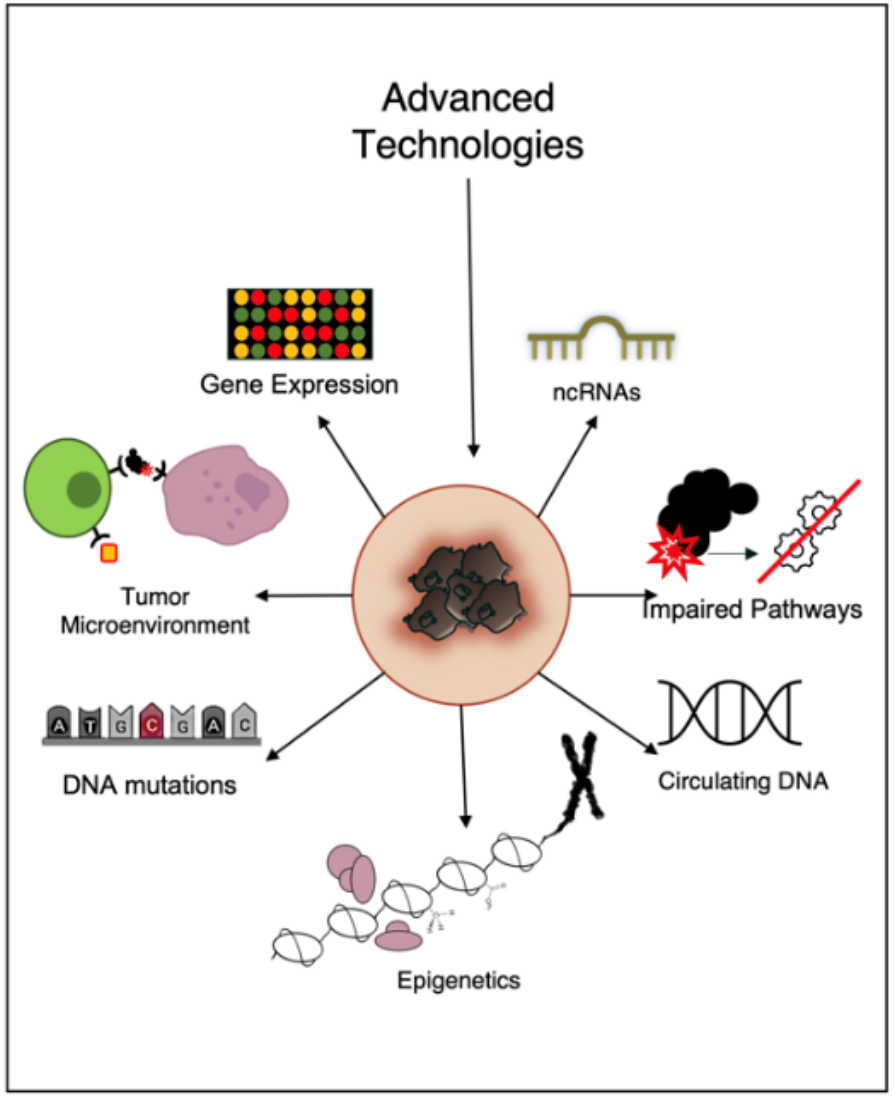
Immunotherapies
Reactivate the cancer–immunity cycle to fight tumour cells[2–4]



▼ This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions.

1. Ribas A, et al. Clin Cancer Res 2012;18:336–341; 2. McArthur GA, Ribas A. J Clin Oncol 2013;31:499–506; 3. Chen DS, Mellman I. Immunity 2013;39:1–10; 4. Drake CG, et al. Nat Rev Clin Oncol. 2014;11:24–37.

Figure 2. The complex realm of biomarkers in melanoma.



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Biomarkers

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MARKER	SENSIBILITA' (%)	SPECIFICITA' (%)
S100B	>93	Bassa
Pmel-17/gp100	>70	>90
MART-1/MelanA	>85	>95
Tyrosinase	>80	Bassa
MITF	>80	Bassa
SOX10	>95	Bassa

Ricerca di biomarker affidabili

Emerging prognostic biomarkers in advanced cutaneous melanoma: a literature update.
Roccuzzo G, Bongiovanni E, Tonella L, Pala V, Marchisio S, Ricci A, Senetta R, Bertero L, Ribero S, Berrino E, Marchiò C, Sapino A, Quaglino P, Cassoni P.
Expert Rev Mol Diagn. 2024 Jan-Feb;24(1-2):49-66. doi: 10.1080/14737159.2024.2314574. Epub 2024 Feb 9.

Biopsia liquida

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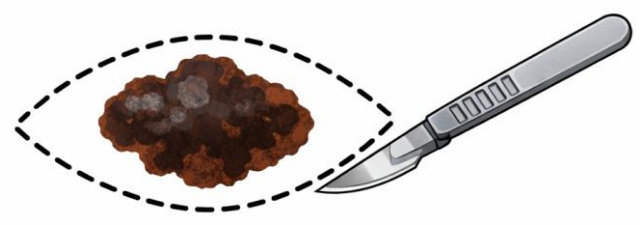
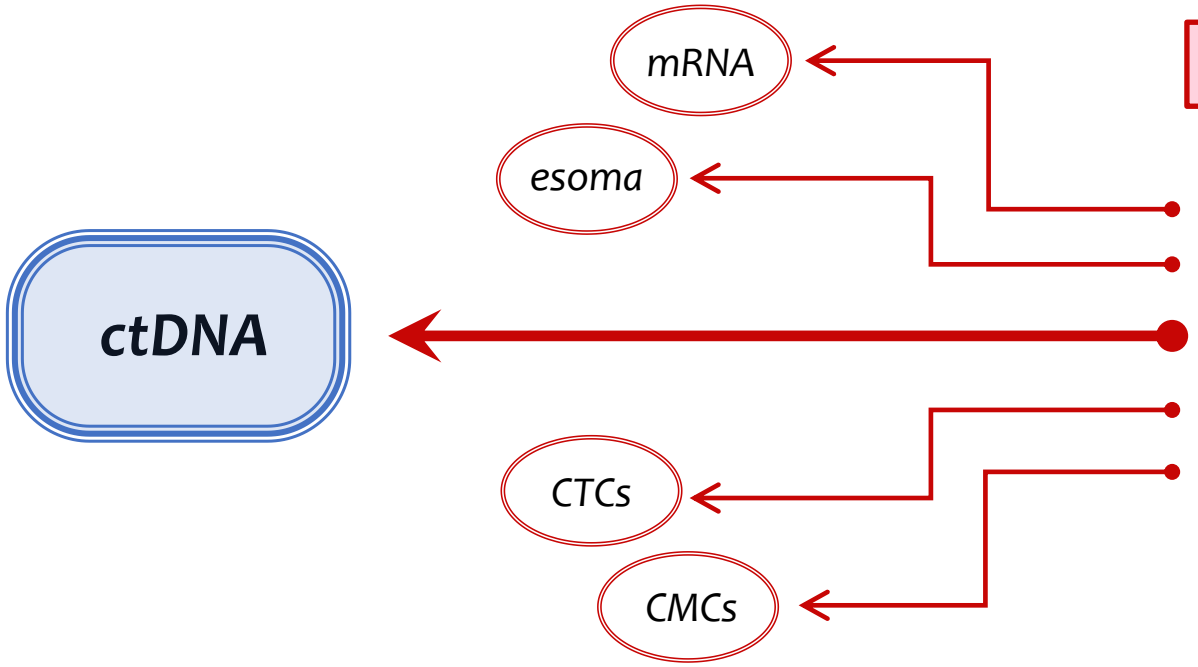
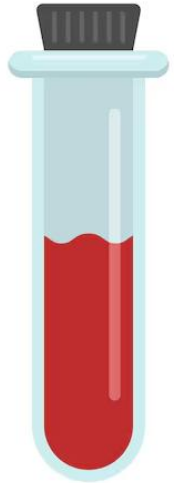
BIOPSIA ESCISSIONALE



- Gold standard diagnosi
- ✗ **Non replicabile**
- ✗ Non utile per monitoraggio

BIOPSIA LIQUIDA





- Non invasivo
- Semplice esecuzione
- A basso costo
- **Replicabile**

Emerging prognostic biomarkers in advanced cutaneous melanoma: a literature update.
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Table 2. Circulating tumor products (CTPs)

Author	Journal	Year	Technique	Biomarker	Analyzed cohort of patients and samples	Main findings
Venturella	*International Journal of molecular science*	2023	NanoAnalyzer, ddPCR	CA-IX associated to sEVs	Metastatic BRAF-positive melanoma patients	CA-IX hypoxia biomarker associated to the membrane of melanoma-derived sEVs, released under hypoxia conditions. Association between overexpression of CA-IX and an aggressive behavior in different types of tumors.
Zeynep Eroglu	*Cancer*	2023	NGS	ctDNA	69 stage III/IV melanoma patients	Increasing ctDNA predictive of shorter DMFS and shorter PFS.
NAHO FUJII	*Anticancer research*	2023	Western blot, IHC	CAF-derived exosomes	90 malignant melanoma	Inhibitory effect on the proliferation of malignant melanoma cells of CD9-positive exosomes. CD9 expression in CAFs as promising prognostic marker for melanoma patients.
Ruggiero	*Theragnostic*	2022	qRT-PCR	6 miRNAs (miR-9-5p, miR-4443, miR-4488, miR-199b-5p, miR-204-5p, miR-579-3p)	70 BRAF-mutant melanoma patients	Association between higher circulating levels of miR-4488 and PD. Association between higher levels of miR-579-3p and disease control (CR, PR, SD).
Albrecht LJ	*Wiley*	2022	ddPCR	cfRNA: KPNA2, DTL, BACE2, DTYMK	361 plasma sample from 100 melanoma patients	Association between high baseline levels of KPNA2 and shorter PFS and OS. Association between high baseline levels of DTL and shorter PFS but not OS. Association between high baseline levels of BACE2 and DTYMK and shorter OS, but not PFS. Increased level of KPNA2, DTL, BACE2, DTYMK copies during therapy in non-responders predictive of shorter PFS. Higher cfRNA detectable in patients with high TMB.
Mattila KE	*Acta Oncologica*	2022	ddPCR	ctDNA	19 metastatic melanoma patients	Association between higher pretreatment ctDNA levels and inferior OS and between detectable ctDNA levels at the time of BOR and inferior PFS and OS in metastatic melanoma patients treated with chemo-immunotherapy ± vemurafenib. Better disease control rate in patients with undetectable pretreatment ctDNA levels
Pietrowska	*Journal of Extracellular Vescicles*	2021	High-resolution mass spectrometry	sEV and anti-CSPG4 antibodies	15 melanoma patients' plasma	Identification of melanoma cell-derived proteins discriminating patients with progression from those with non-evident or stable disease after therapy.
Wu X	*Hindawi genetic research*	2022	linear models for microarray data	miRNA: Hsa-let-7c-5p, hsa-miR-130b-3p, and hsa-miR-142-3p	Melanoma patients from GEO database	Association between miRNA hsa-let-7c-5p, hsa-miR-130b-3p, hsa-miR- 142-3p, and hsa-miR-509-3p and prognosis in melanoma patients. Hsa-miR-130b-3p overexpression is associated with poorer survival.
Tawbi HA	*Journal for Immunotherapy of Cancer*	2022	IHC, RNA-seq, DNA-seq, flow cytometric, Nanostring	T-cell inflammation, ctDNA, CD4+/CD8+ t-cell ratio	Tumor tissue and blood from 532 stage IIIC/IV melanoma patients	T-cell inflammation as a prognostic marker of spartalizumab + Dabrafenib/Trametinib benefit. Baseline ctDNA shedding as a strong independent prognostic variable. CD4+/ CD8+ T-cell ratio as a predictive marker of PFS benefit with spartalizumab+Dabrafenib/Trametinib.
Varaljai	*Journal fo the European academy of dermatology and venereology*	2021	Qubit® platform -DNA spectrophotometry; RNase P qPCR	cfDNA	Plasma samples in a discovery cohort (n = 20), expansion cohort, (n = 166) of metastatic melanoma patients and healthy donors (n = 116)	Association of high cfDNA with presence of metastases, higher stage, shorter OS.

Legend to Table 2. BOR: best objective response; CAF: cancer-associated fibroblasts; cfDNA: cell-free DNA; cfRNA: cell-free RNA CR: complete response; ctDNA: circulating tumor DNA; DCR: disease control rate; ddPCR: droplet digital PCR; DNA-seq: DNA-sequencing; GEO database: gene expression omnibus database; miRNAs: microRNAs; NGS: next generation sequencing; sEVs: small extracellular vesicles; DMFS: distant metastasis-free survival; PFS: progression-free survival; IHC: immunohistochemistry; OS: overall survival; PD: progressive disease; PFS: progression-free survival; PR: partial response; qRT-PCR: quantitative real-time PCR; RNA-seq: RNA-sequencing; SD: stable disease; TMB: tumor mutation burden.

Heidrich *et al. J Exp Clin Cancer Res* (2025) 44:202
https://doi.org/10.1186/s13046-025-03457-w

Journal of Experimental &
Clinical Cancer Research

RESEARCH Open Access

In-depth assessment of BRAF, NRAS, KRAS, EGFR, and PIK3CA mutations on cell-free DNA in the blood of melanoma patients receiving immune checkpoint inhibition

Isabel Heidrich[1,2,3*], Charlotte Rautmann[1,2], Cedric Ly[4,5,6,7], Robin Khatri[5,6,7], Julian Kött[2], Glenn Geidel[2], Alessandra Rünger[2], Antje Andreas[1], Inga Hansen-Abeck[2], Finn Abeck[2], Anne Menz[8], Stefan Bonn[5,6,7], Stefan W. Schneider[2], Daniel J. Smit[1†], Christoffer Gebhardt[2†] and Klaus Pantel[1,9*†]

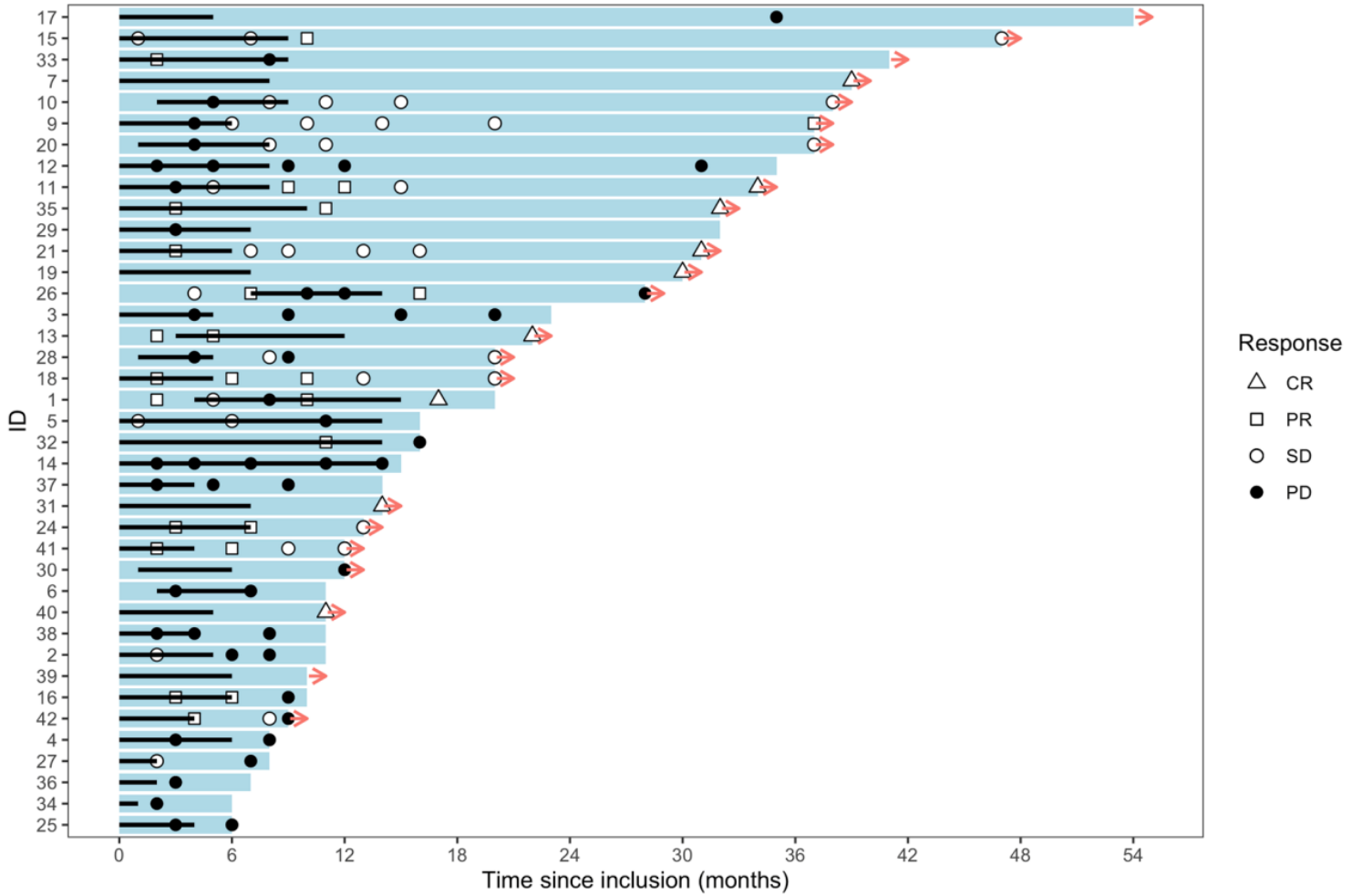

Fig. 5 Swimmer plot showing the clinical course of all 39 patients included in the study. Each horizontal bar represents one patient and illustrates the duration of study inclusion. Arrows at the end of bars indicate patients who were still alive at the last follow-up. Black lines indicate the time frame in which ctDNA measurements were conducted. Radiological responses assessed by MRI (brain) and CT (thorax, abdomen, pelvis) every 3–4 months are indicated by different symbols: △ complete remission (CR), □ partial remission (PR), ○ stable disease (SD), and ● progressive disease (PD). The black bars represent longitudinal ctDNA measurements

www.oncotarget.com Oncotarget, 2018, Vol. 9, (No. 90), pp: 36238-36249

Research Paper

Monitoring BRAF and NRAS mutations with cell-free circulating tumor DNA from metastatic melanoma patients

Elodie Long-Mira[1,2,3,*], Marius Ilie[1,2,3,*], Emmanuel Chamorey[4], Florence Leduff-Blanc[5], Henri Montaudié[5], Virginie Tanga[3], Maryline Allégra[3], Virginie Lespinet-Fabre[3], Olivier Bordone[3], Christelle Bonnetaud[3], Renaud Schiappa[4], Catherine Butori[1], Coraline Bence[1], Jean-Philippe Lacour[5], Véronique Hofman[1,2,3] and Paul Hofman[1,2,3]

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[*]These authors contributed equally to this work

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Keywords: *metastatic melanoma; BRAF; NRAS; cfDNA; IDYLLA™*

Received: *July 19, 2018* ***Accepted:*** *November 01, 2018* ***Published:*** *November 16, 2018*

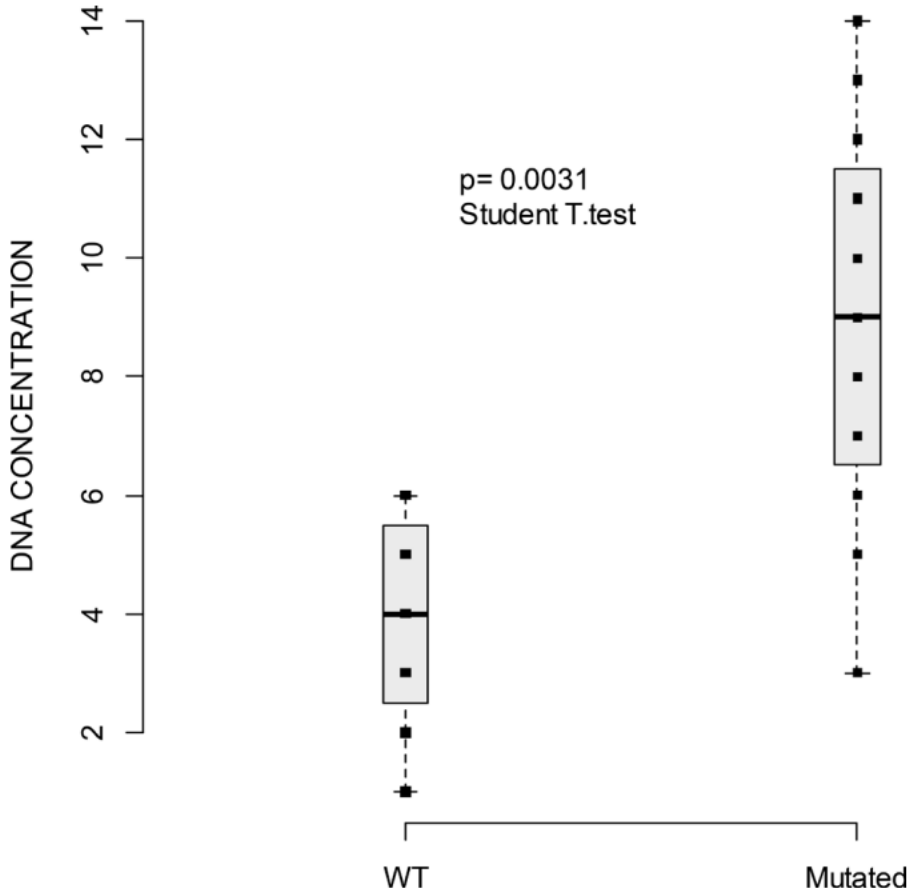
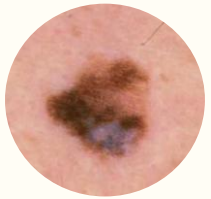


Figure 2: Boxplot of cfDNA concentration (measured with Qubit, ng/µl) and the presence of plasmatic mutations WT versus mutated. Horizontal line indicates median = 4 ng/µL (range: 1–6) for WT cfDNA [range 0.6 to 390.0 ng/µL] and median = 9 ng/µL (range: 3–14) for mutated cfDNA. Squares indicate the value of cfDNA. *P*-value of the Student *T*-test indicates a significant difference of cfDNA mean concentration between WT and mutated.

BACKGROUND: CUTANEOUS MELANOMA



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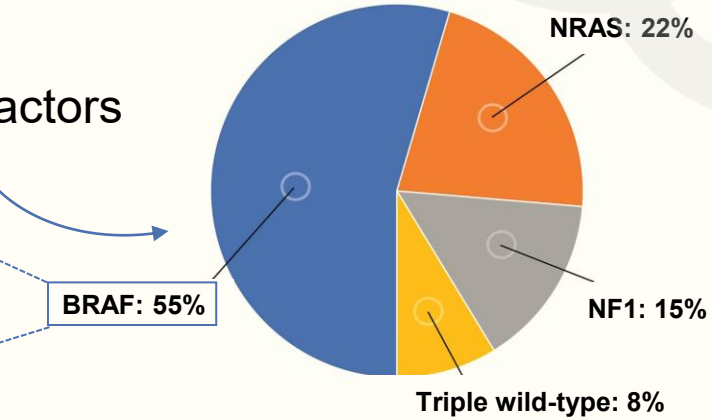
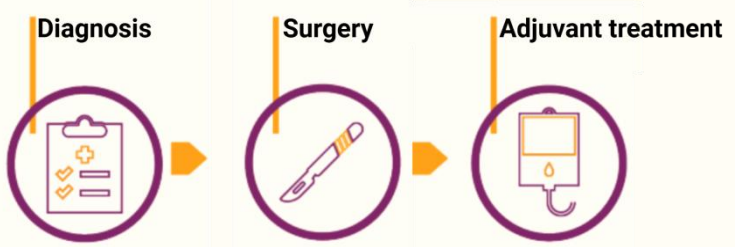


FDA

- BRAF-mutated patients: targeted therapy (**TT**) BRAF plus MEK inhibitors (*Dabrafenib+Trametinib)*
- BRAF-WT patients*:* immune checkpoint inhibitors (**ICI**) (e.g. anti-PD-1)

Resected stage III/IV CM



- Short-term benefit
- High cost and toxicities

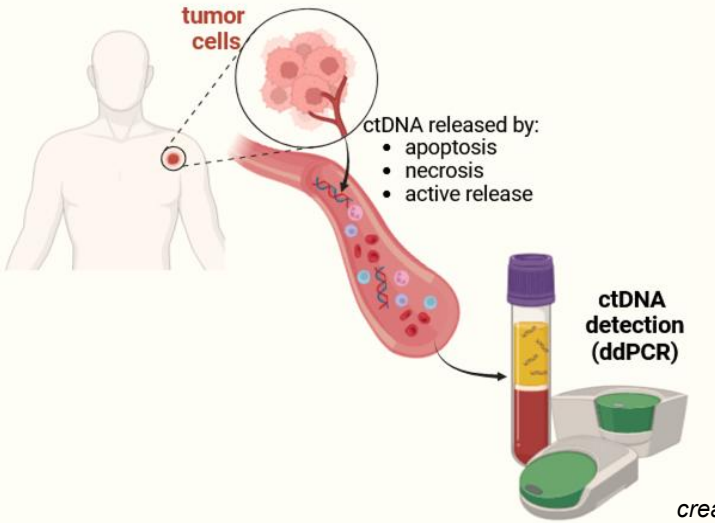
There is a urgent need for robust biomarkers capable of identifying high-risk patients and monitoring disease course in stage III CM

Images adapted from Florent L., Cancers, 2023 ; Ortiz-Urda S., et al, Melanoma, 2024

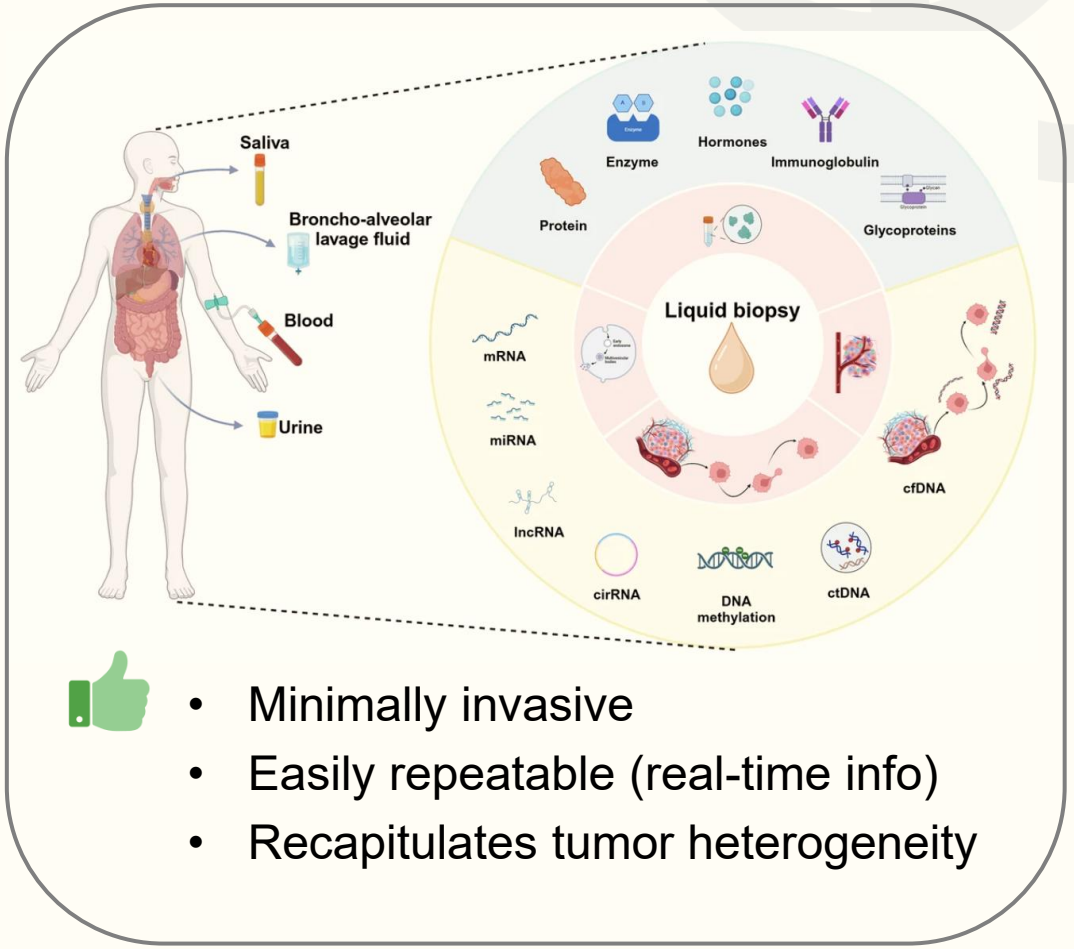
TOWARDS NOVEL BIOMARKERS: ctDNA

Circulating tumor DNA (**ctDNA**) is the **tumor-derived fraction** of circulating free DNA

- Directly assesed in blood samples
- Retains genetic/epigenetic alterations of the tumor
- Technically challenging detection (0.1-5% of cfDNA): requires highly sensitive and standardized methods
- Confirmed **prognostic relevance in metastatic CM**



created with Biorender.com



- Minimally invasive
- Easily repeatable (real-time info)
- Recapitulates tumor heterogeneity

Limited studies on the utility of post-operative ctDNA in the adjuvant settings in CM

Image adapted from Ma L, et al. Sig Transduct Target Ther 2024; Michaelidou K., et al. Cells 2020

Can ctDNA serve as a clinically meaningful biomarker for risk stratification and treatment monitoring in resected stage III CM patients receiving adjuvant therapy?

1. **Assess the prognostic value of post-operative ctDNA detection in stage III CM patients**

2. **Explore the potentiality of longitudinal ctDNA evaluation to monitor disease course and relapse during adjuvant therapy**

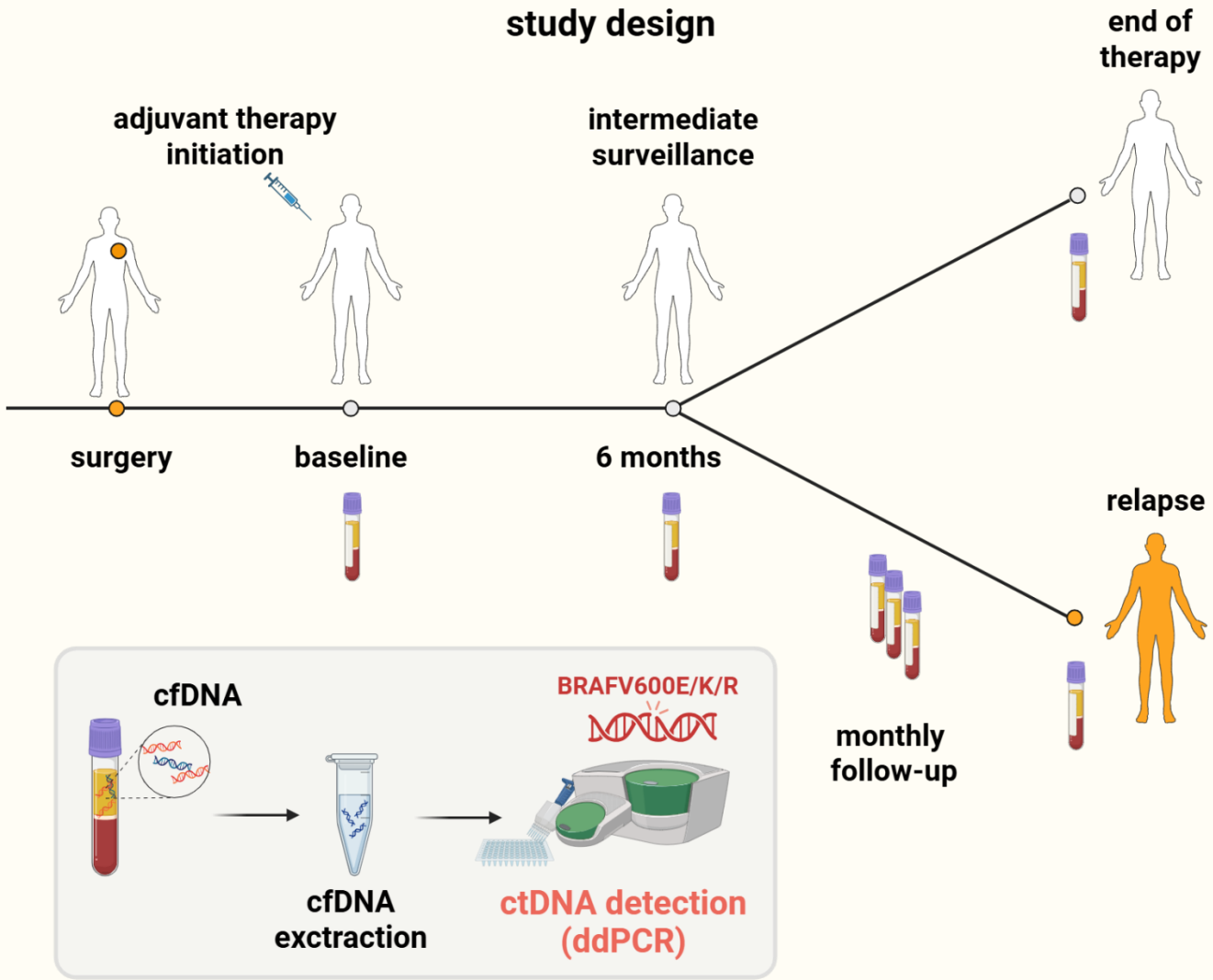
WORKFLOW

patient enrollement (2019-2021)

- Stage IIIA-D CM
- Confirmed BRAF mutation
- Fully resected melanoma w/ distant metastasis
- Adjuvant therapy (TT/ICI)

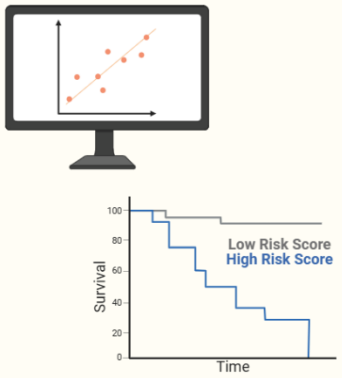
CM patients (n=32)		
Sex	Male	15 (46.9%)
	Female	17 (53.1%)
Age at diagnosis (years)	Median	50
	Interval	32-80
Stage	IIIA	3 (9.4%)
	IIIB	9 (28%)
	IIIC	18 (56.3%)
	IIID	2 (6.3%)
Tumor site	Limbs	11 (34.4%)
	Trunk	19 (59.4%)
	Head/neck	2 (6.2%)
Adjuvant therapy	TT	29 (90.6%)
	ICI (anti-PD-1)	3 (9.4%)
Histotype	SSM	23 (71.9%)
	NM	6 (18.8%)
	AM	2 (6.3%)
	Missing	1 (3%)

study design

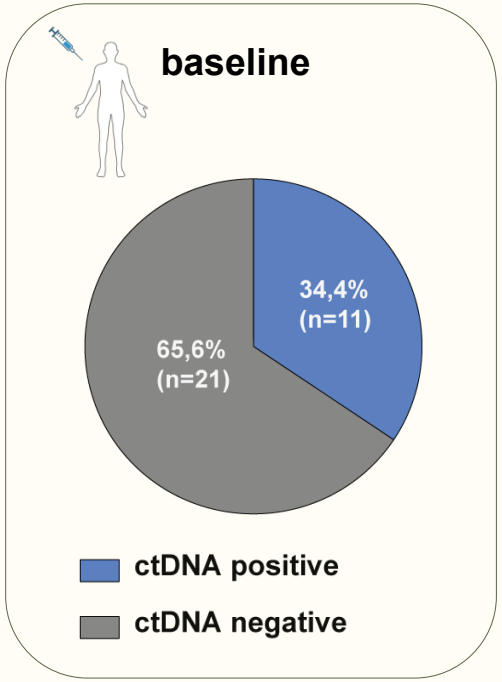


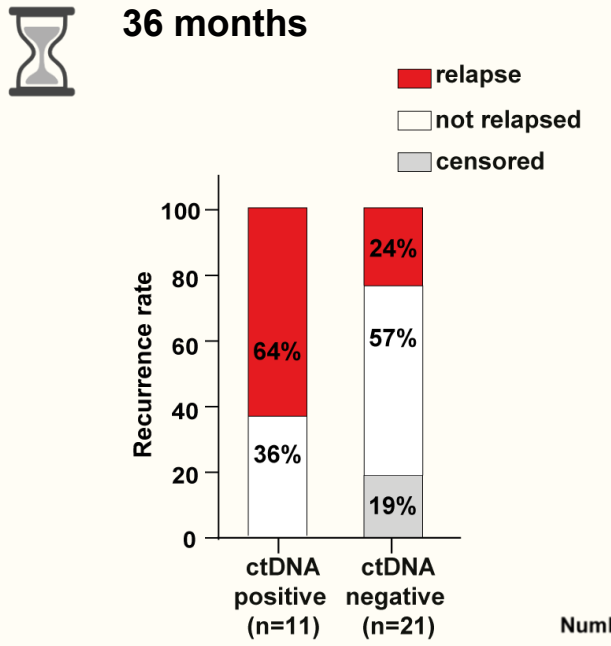
Comparison with clinical data

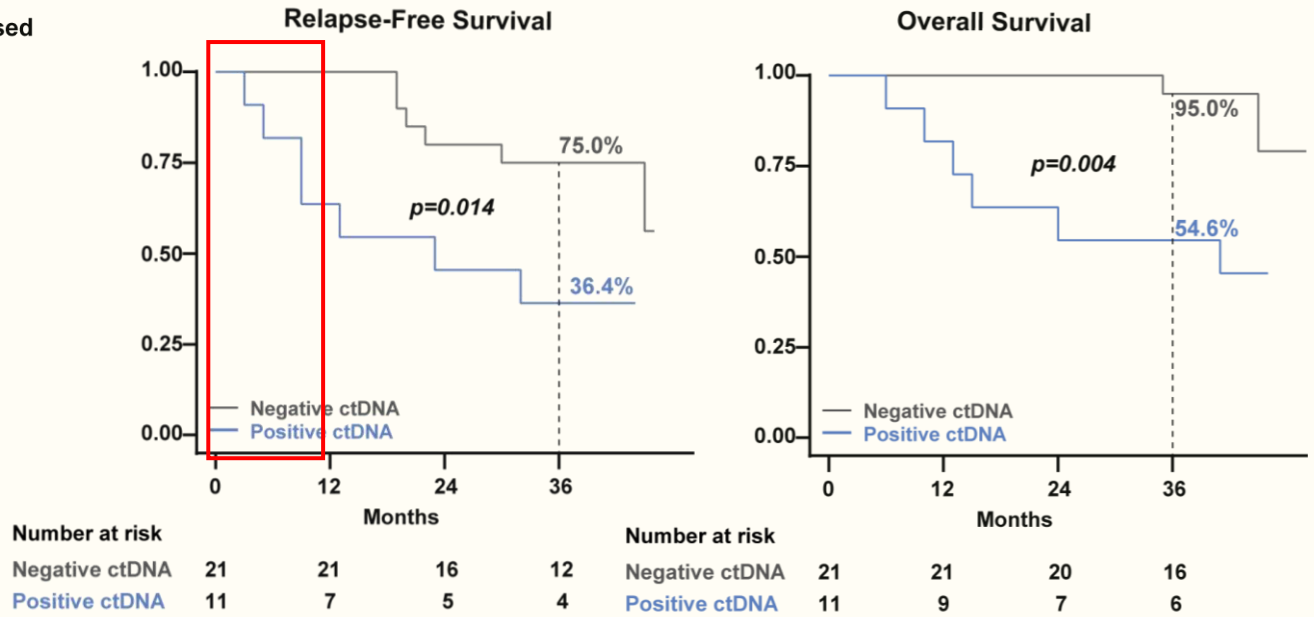
36 months



1 Baseline ctDNA detection (ctDNA+) identifies high-risk CM patients



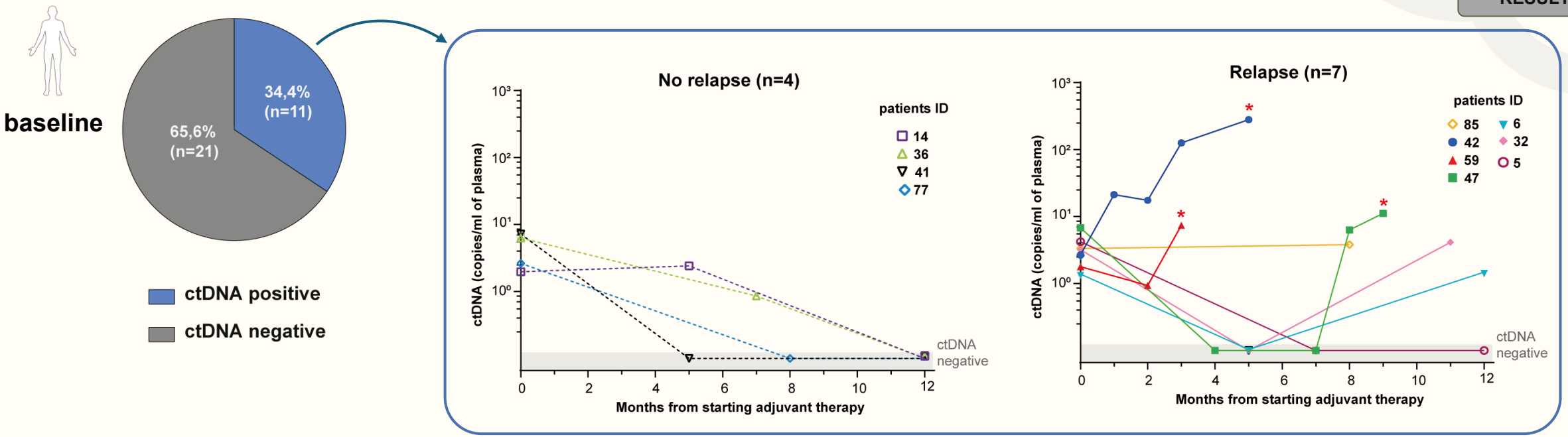




- **ctDNA+** patients show **higher incidence** of experiencing disease relapse compared ctDNA- patients
- **Baseline ctDNA detection** was associated with an **increased risk of relapse** and **poorer survival**
- All patients **relapsing within 1-year adjuvant treatment** were in the **ctDNA+ group (high-risk)**

Marchisio S. et al, J Transl Med, 2024

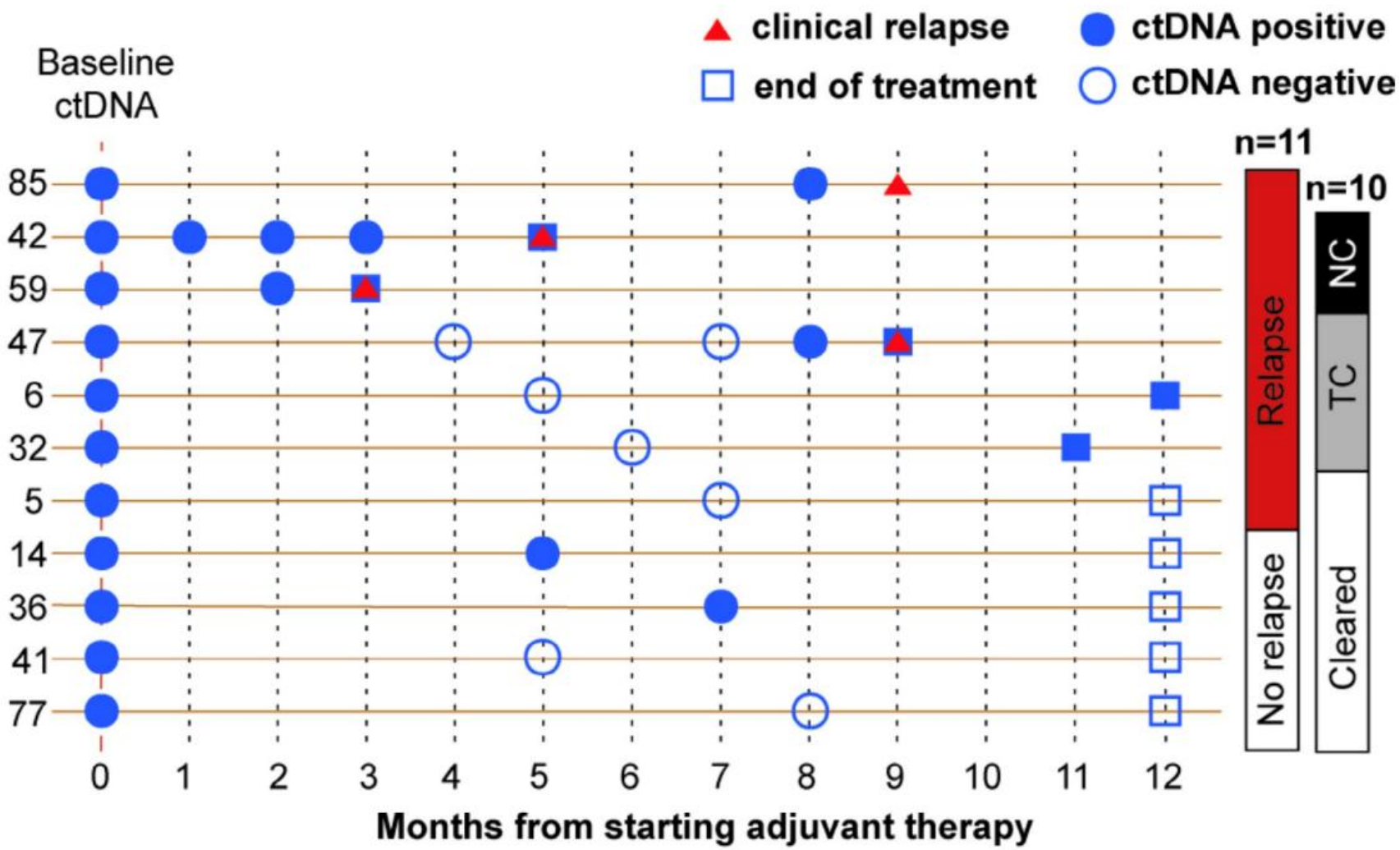
2 Failure to clear plasma ctDNA during adjuvant therapy likely predicts disease relapse



- **In disease-free patients** plasma **ctDNA became undetectable** within one year of monitoring
- All patients with **longitudinal ctDNA persistence** (or transient clearance) **relapsed within 36 months**

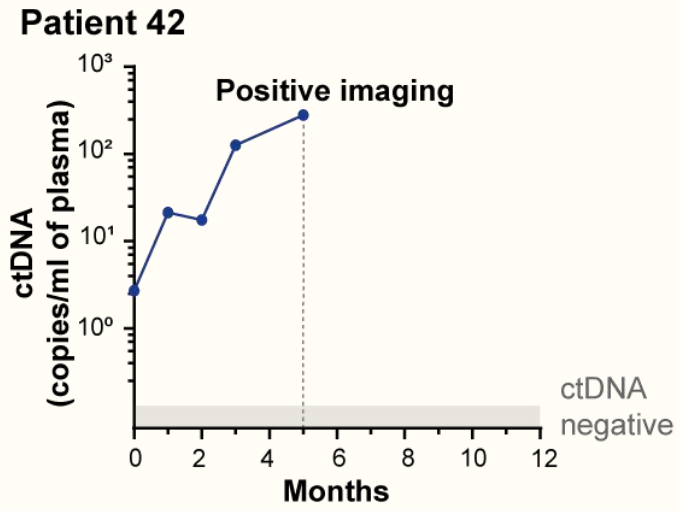
Marchisio S. et al, J Transl Med, 2024

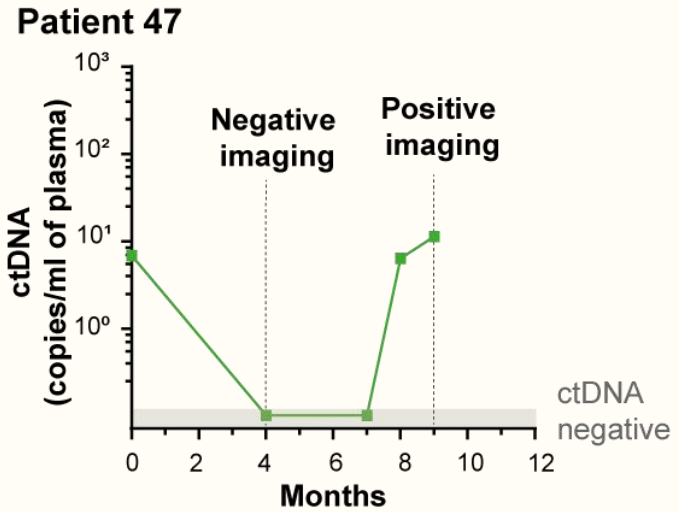
a

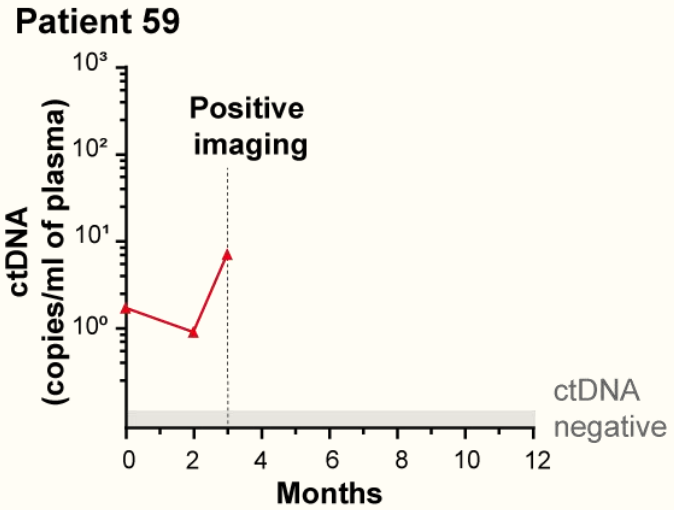

clinical relapse
ctDNA positive
end of treatment
ctDNA negative
Baseline ctDNA
85
42
59
47
6
32
5
14
36
41
77
0
1
2
3
4
5
6
7
8
9
10
11
12
Months from starting adjuvant therapy
n=11
n=10
Relapse
No relapse
NC
TC
Cleared

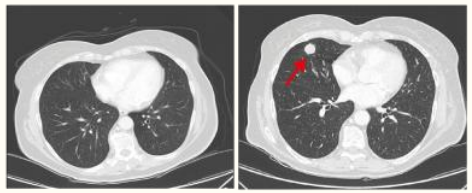
b

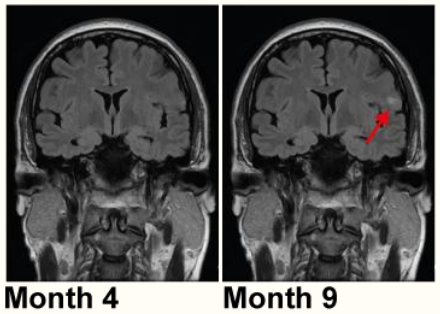
2 Serial ctDNA analysis reveals early relapse during adjuvant therapy

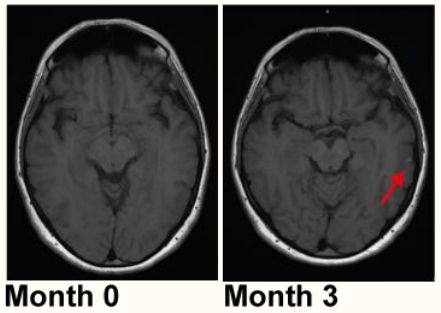












- Patients who **relapsed** during **adjuvant treatment** showed **higher ctDNA levels** compared to patients with longer RFS (> 1 year), with ctDNA anticipating the clinical evidence of relapse

Marchisio S. et al, J Transl Med, 2024

CONCLUSIONS

- Postoperative ctDNA positivity was associated with an increased risk of relapse and poorer survival in stage III CM, supporting ctDNA clinical utility as prognostic biomarker;

ctDNA-guided risk stratification may help adjuvant treatment decisions, sparing low-risk patients from unnecessary therapy

- During adjuvant therapy persistent ctDNA positivity likely predicts disease recurrence, with longitudinal ctDNA monitoring anticipating radiological evidence of relapse;

ctDNA monitoring during adjuvant therapy represents a surrogate marker of treatment response

- Small sample size
- Short plasma sampling window
- Low number of recurrence events

ddPCR-based ctDNA detection is a non-invasive, cost-effective and real-time surveillance tool suitable for integration into routine clinicopathological workflow

Can ctDNA serve as a clinically meaningful biomarker for risk stratification and treatment monitoring in resected stage III CM patients receiving adjuvant therapy?

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RESEARCH

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Monitoring circulating tumor DNA liquid biopsy in stage III BRAF-mutant melanoma patients undergoing adjuvant treatment

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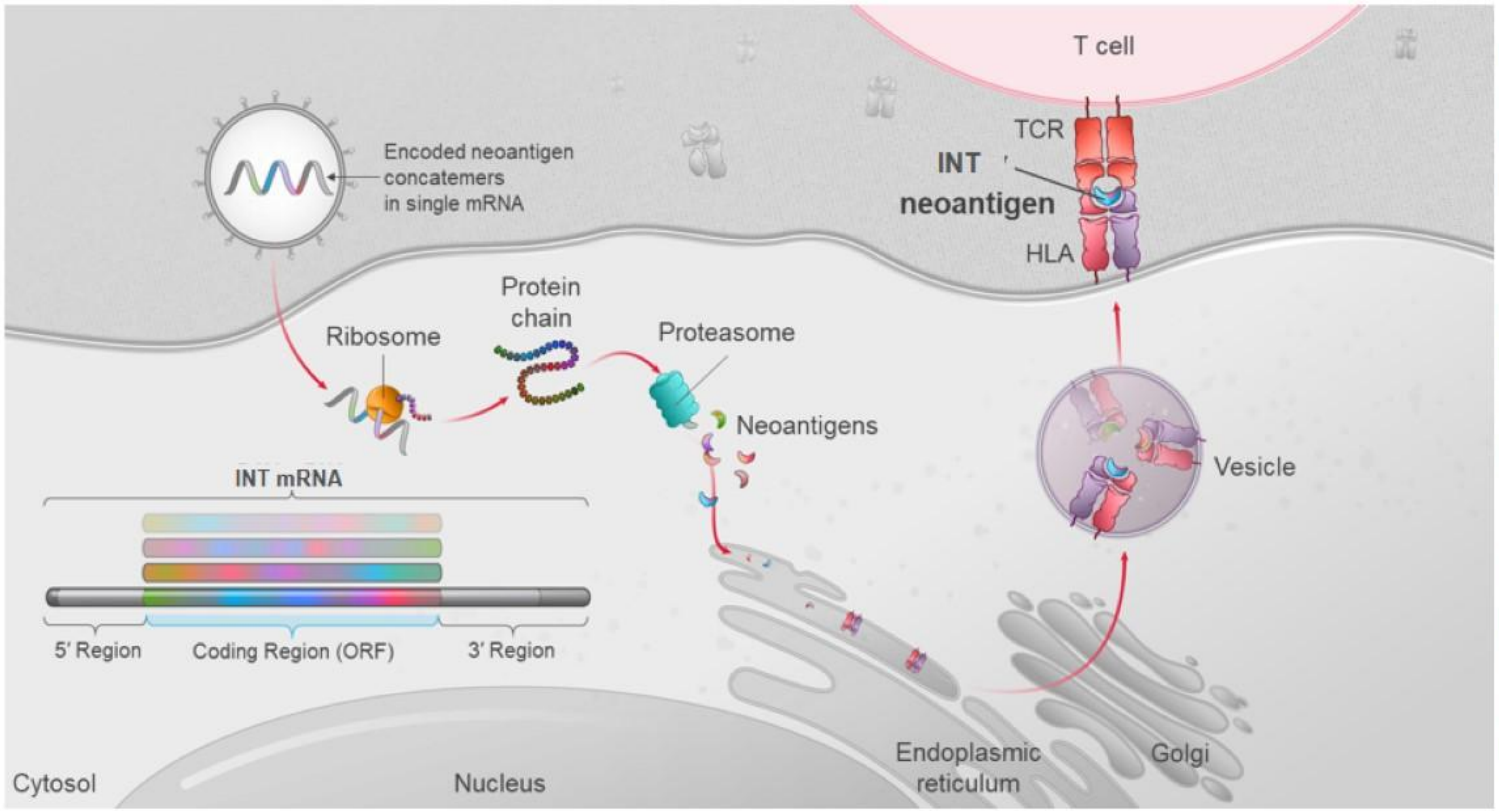
mRNA-4157 (V940) Mechanism of Action



mRNA-4157 (V940) is a **customizable** individualized neoantigen therapy encoding up to 34 neoantigens

Targeting of neoantigens by T-cells has been demonstrated to **drive antitumor responses**[1]

The modified mRNA **platform** was implemented for the COVID-19 vaccine (mRNA-1273), demonstrating its **utility and adaptability**[2]

HLA, human leukocyte antigen; mRNA, messenger RNA; ORF, open reading frame; PCV, personalized cancer vaccine; RFS, recurrence-free survival; TCR, T-cell receptor.

1. Wirth TC, Kühnel F. *Front Immunol*. 2017;8:1848. 2. Baden LR, et al. *N Engl J Med*. 2021;384(5):403-416.

Proprietary

A Personalized Cancer Vaccine, mRNA-4157 (V940), Combined With Pembrolizumab Versus Pembrolizumab Alone in Patients With Resected High-risk Melanoma: Efficacy and Safety Results From the Randomized, Open-label Phase 2 mRNA-4157-P201/KEYNOTE-942 Trial

Adnan Khattak,[1,2] Matteo Carlino,[3] Tarek Meniawy,[4] George Ansstas,[5] Theresa Medina,[6] Matthew H. Taylor,[7] Kevin B. Kim,[8] Meredith McKean,[9] Georgina V. Long,[10] Ryan J. Sullivan,[11] Mark Faries,[12] Thuy Tran,[13] Charles Cowey,[14] Andrew Pecora,[15] Jennifer Segar,[16] Victoria Atkinson,[17] Geoffrey T. Gibney,[18] Jason Luke,[19] Sajeve Thomas,[20] Elizabeth Buchbinder,[21] Peijie Hou,[22] Lili Zhu,[22] Tal Zaks,[22] Michelle Brown,[22] Praveen Aanur,[22] Robert S. Meehan,[22] Jeffrey S. Weber[24,*]

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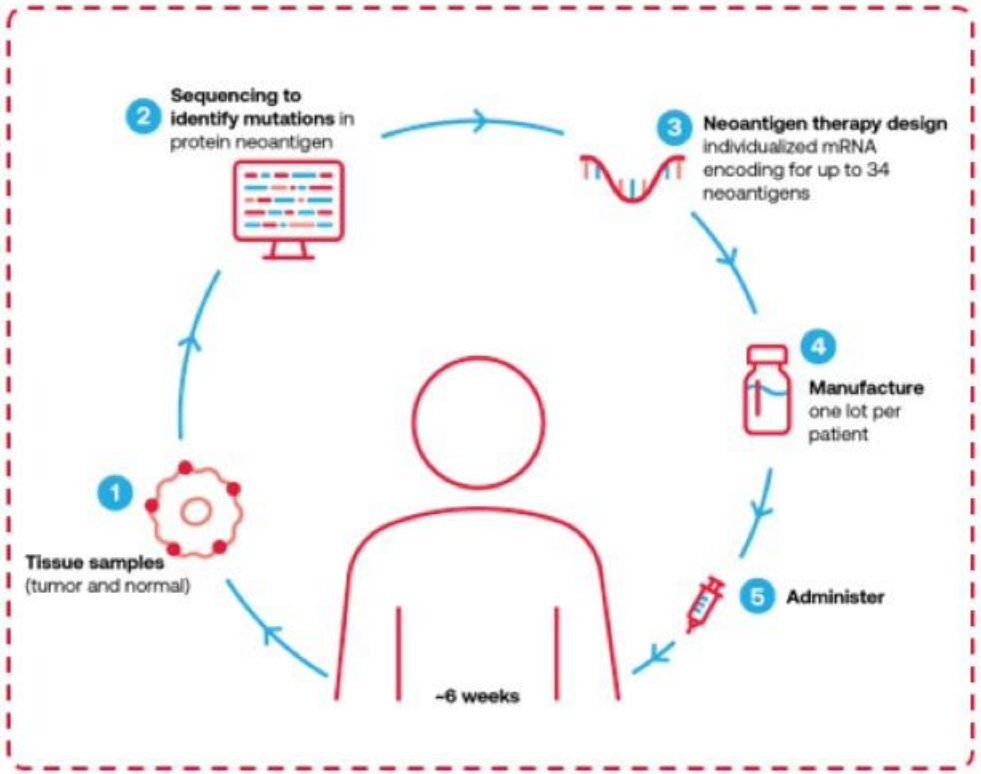
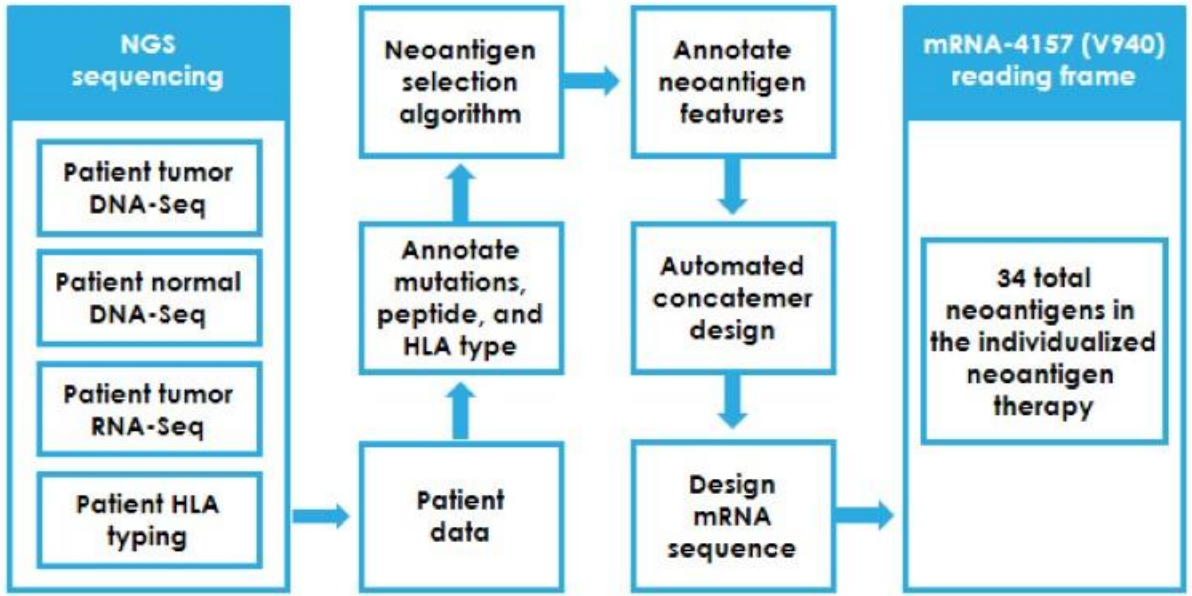
Sponsored by Moderna, Inc., in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.

mRNA-4157 (V940): An Individualized Neoantigen Therapy (INT)

Designed to target an individual patient's unique tumor mutations through integrated manufacturing





DNA-Seq, DNA sequencing; HLA, human leukocyte antigen; mRNA, messenger RNA; NGS, next-generation sequencing; RNA-Seq, RNA sequencing.

mRNA-4157-P201/KEYNOTE-942 (NCT03897881) Study Design

Randomized, phase 2, open-label study in adjuvant resected melanoma patients at high risk of recurrence

Key eligibility criteria
- Stage IIIB,[a] IIIC, IIID, or IV cutaneous melanoma
- Complete surgical resection within 13 weeks prior to first pembrolizumab dose
- Disease-free at study entry
- ECOG PS 0-1
- Tissue available for NGS

2:1 Randomization

Stratified by disease stage[b]

Combination treatment arm: mRNA-4157 (V940) + pembrolizumab
Up to 1 year of pembrolizumab treatment
mRNA-4157 (V940) 1 mg IM Q3W for up to 9 doses +
pembrolizumab 200 mg IV Q3W for up to 18 cycles
(n = 107)

Control treatment arm: pembrolizumab only
Up to 1 year of pembrolizumab treatment
Pembrolizumab 200 mg IV Q3W for up to 18 cycles
(n = 50)

Primary endpoint:
RFS[c,d]

Secondary endpoints:
DMFS,
Safety, tolerability

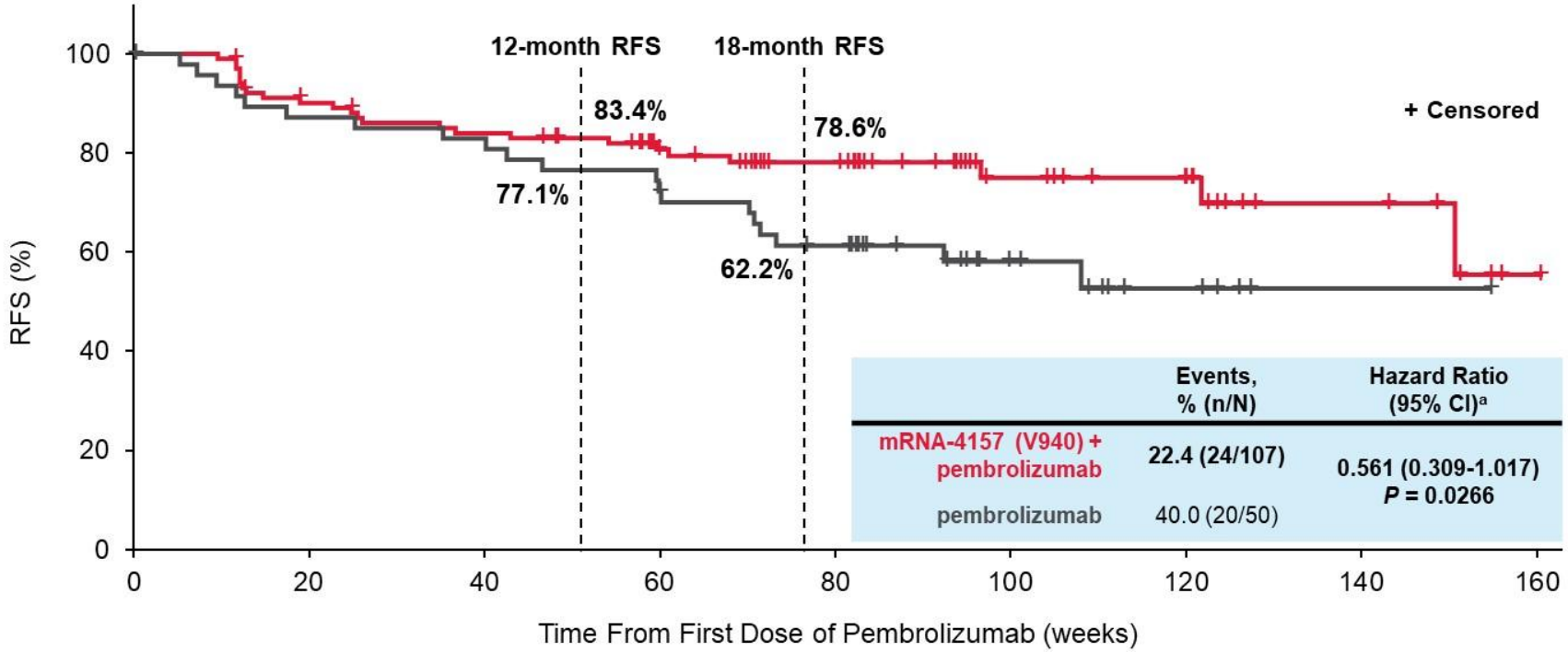
RFS follow-up: up to 3 years following the first dose of pembrolizumab

Designed with 80% power to detect an HR of 0.5 with ≥40 RFS events (with 1-sided alpha of 0.1)

Median follow-up[e]: 23 months for mRNA-4157 (V940) + pembrolizumab
24 months for pembrolizumab only

DMFS, distant metastasis-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; IM, intramuscular; IV, intravenous; mRNA, messenger RNA; NGS, next-generation sequencing; Q3W, every 3 weeks; RFS, recurrence-free survival.
[a]Patients with stage IIIB disease were eligible only if relapse occurred within 3 months of prior surgery of curative intent. [b]According to the 8th edition of the American Joint Committee on Cancer staging manual. [c]The primary endpoint was investigator-assessed RFS (defined as the time from first dose of pembrolizumab until the date of first recurrence [local, regional, or distant metastasis], a new primary melanoma, or death from any cause) in the intention-to-treat population. [d]The primary analysis for RFS was specified to occur after all patients completed ≥12 months on study and ≥40 RFS events were observed. Descriptive analysis specified to occur when ≥51 RFS events observed [e]Time of database cutoff was November 14, 2022.

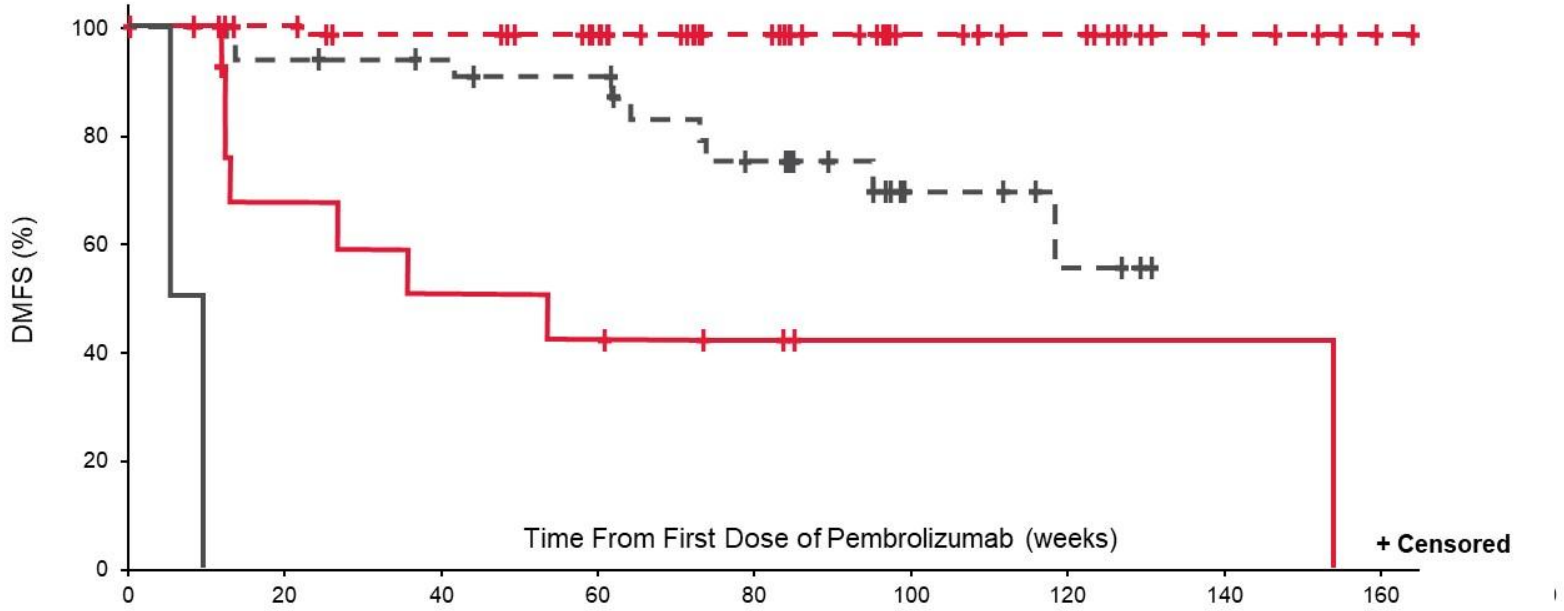
Primary Efficacy Endpoint: RFS[1]



[a]The hazard ratio and 95% CI for mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab is estimated using a Cox proportional hazards model with treatment group as a covariate, stratified by disease stage (stages IIIB or IIIC or IIID vs stage IV) used for randomization. The *P* value is based on a 1-sided log-rank test stratified by disease stage (stages IIIB or IIIC or IIID vs stage IV) used for randomization.

1. Khattak A, et al. Presented at the American Association for Cancer Research® (AACR) Annual Meeting; April 14-19, 2023; Orlando, FL, USA. Oral presentation CT001.

DMFS by ctDNA Status at Baseline



Number at Risk

ctDNA-neg: mRNA-4157 (V940) + pembrolizumab	77	73	69	57	38	18	15	6	1
ctDNA-neg: pembrolizumab	33	30	28	26	18	7	4	0	0
ctDNA-pos: mRNA-4157 (V940) + pembrolizumab	13	8	6	5	3	1	1	1	0
ctDNA-pos: pembrolizumab	2	0	0	0	0	0	0	0	0

	mRNA-4157 (V940) + pembro vs pembro HR (95% CI)[a]	mRNA-4157 (V940) + pembro Events, % (n/N)	pembro Events, % (n/N)
ctDNA-neg	0.048 (0.006, 0.380)	1.3 (1/77)	27.3 (9/33)
ctDNA-pos	NE	61.5 (8/13)	100 (2/2)

See LBA9515 presented by Matteo S. Carlino on 03Jun

Minimal residual disease by circulating tumor DNA as a biomarker of recurrence free survival in resected high-risk melanoma patients treated with mRNA-4157 (V940), a personalized cancer vaccine, and pembrolizumab.

ctDNA was NE at baseline for 20.4% (32/157) patients from this study due to unavailability of the sample at baseline (mRNA-4157 (V940) + pembrolizumab, n = 15; pembrolizumab monotherapy, n = 14) or insufficient number of RaDaR® variants identified by WES (quality control flag: mRNA-4157 (V940) + pembrolizumab, n = 2; pembrolizumab monotherapy, n = 1). Results limited by small sample size and event number. ctDNA, circulating tumor DNA.

Clinical validation of droplet digital PCR assays in detecting *BRAF*^{V600}-mutant circulating tumour DNA as a prognostic biomarker in patients with resected stage III melanoma receiving adjuvant therapy (COMBI-AD): a biomarker analysis from a double-blind, randomised phase 3 trial

Mahrukh M Syeda, Georgina V Long, James Garrett, Victoria Atkinson, Mario Santinami, Dirk Schadendorf, Axel Hauschild, Michael Millward, Mario Mandala, Vanna Chiarion-Sileni, Michael Smylie, Georgy M Manikhas, Reinhard Dummer, Jennifer M Wiggins, Saim Ali, Sachin Bajirao Adnaik, Monique Tan, Maya Dajee, David Polsky

Lancet Oncol 2025; 26: 641–53

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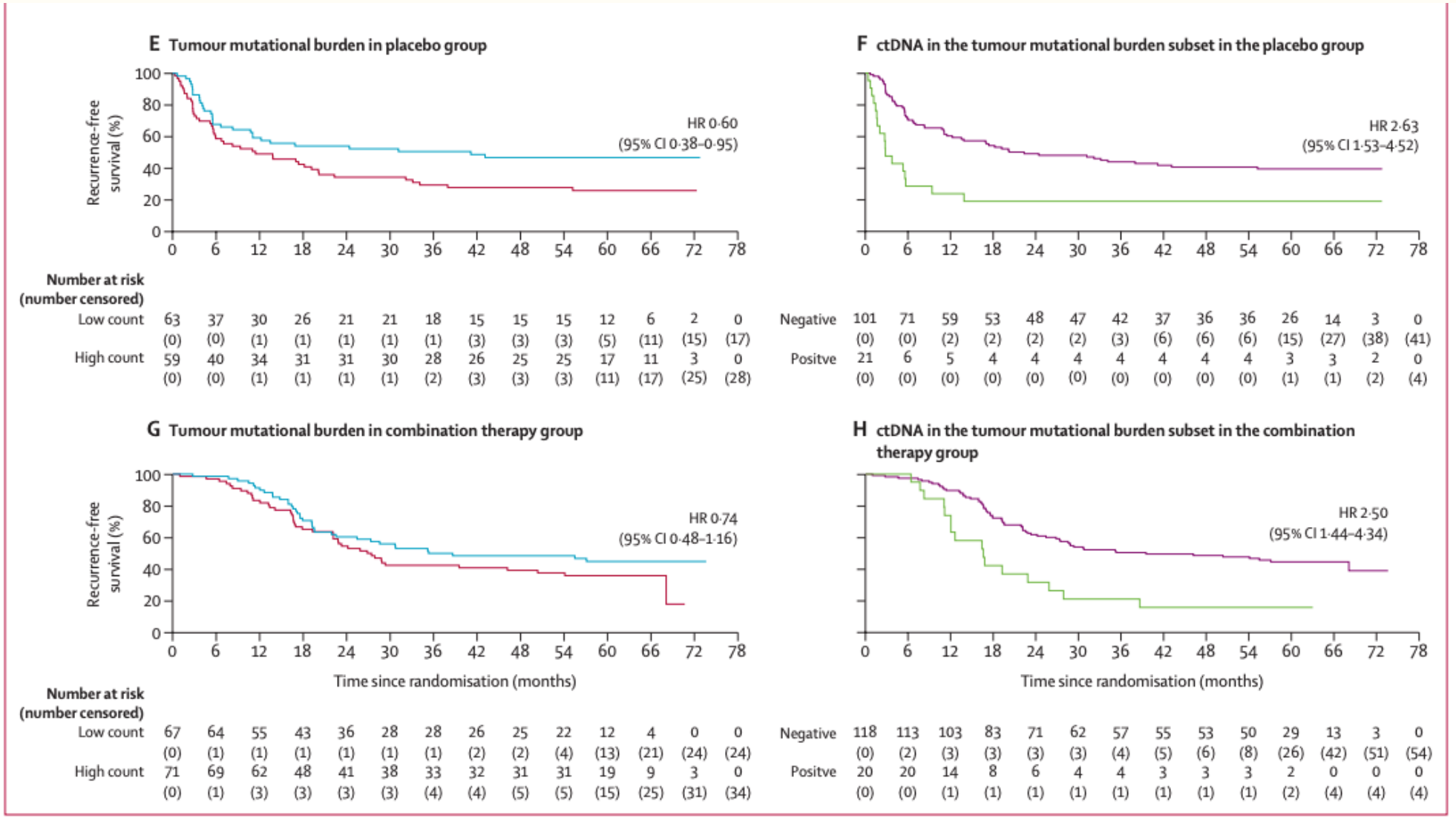
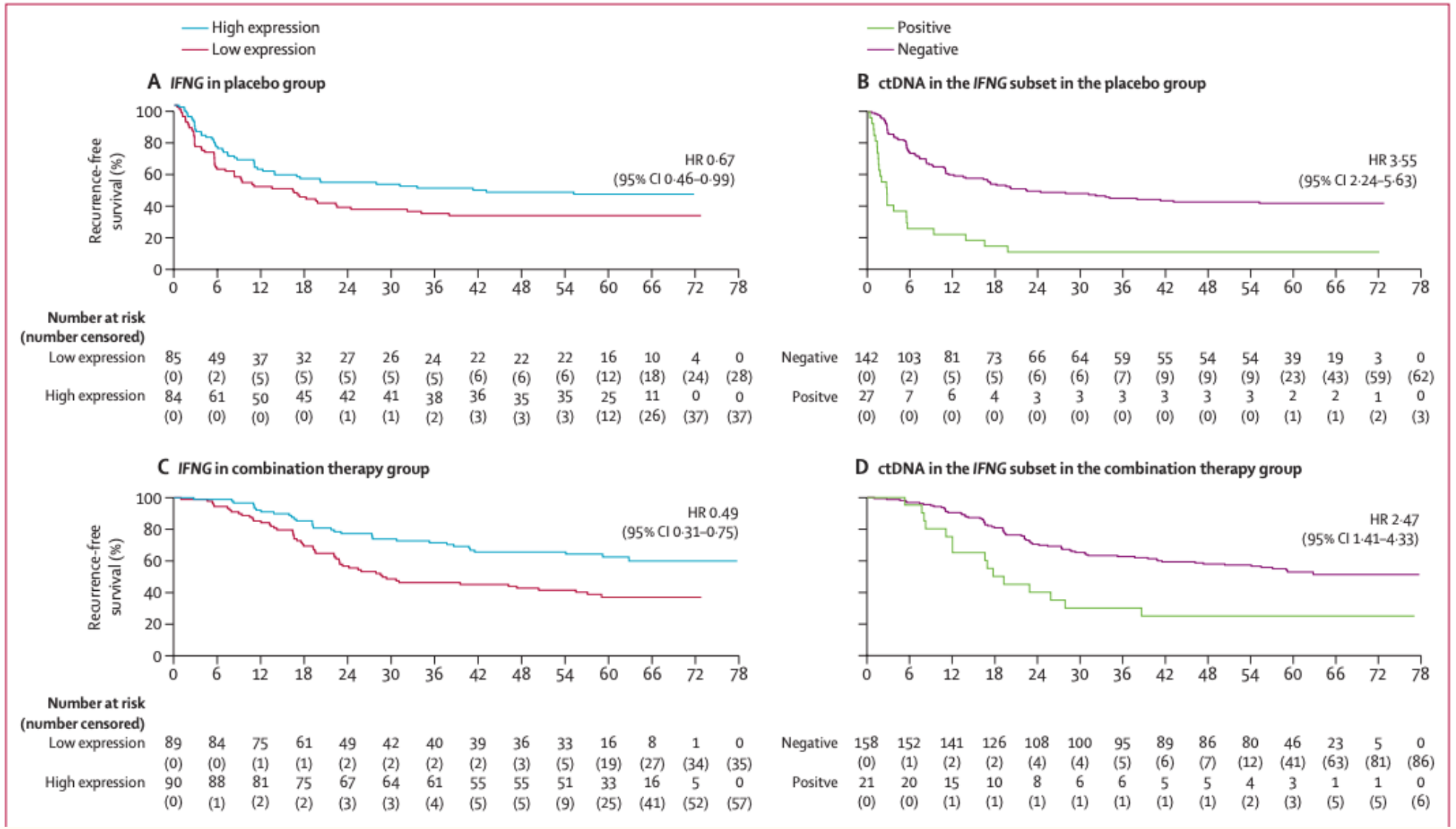
See Comment page 538

Summary

Background Cell-free, circulating tumour DNA (ctDNA) is an established measure of minimal residual disease; however, it is not utilised in melanoma management. We investigated whether ctDNA measurements could predict survival outcomes during adjuvant targeted therapy or placebo treatment in stage III melanoma, thereby identifying patients at high risk and low risk of recurrence.

Methods Analytically validated mutation-specific droplet digital PCR assays were used to measure *BRAF*^{V600E} or


E Tumour mutational burden in placebo group
Recurrence-free survival (%)
HR 0·60
(95% CI 0·38–0·95)
Number at risk (number censored)
Low count 63 37 30 26 21 21 18 15 15 15 12 6 2 0
(0) (0) (1) (1) (1) (1) (1) (3) (3) (3) (5) (11) (15) (17)
High count 59 40 34 31 31 30 28 26 25 25 17 11 3 0
(0) (0) (1) (1) (1) (1) (2) (3) (3) (3) (11) (17) (25) (28)
F ctDNA in the tumour mutational burden subset in the placebo group
HR 2·63
(95% CI 1·53–4·52)
Negative 101 71 59 53 48 47 42 37 36 36 26 14 3 0
(0) (0) (2) (2) (2) (2) (3) (6) (6) (6) (15) (27) (38) (41)
Positve 21 6 5 4 4 4 4 4 4 4 3 3 2 0
(0) (0) (0) (0) (0) (0) (0) (0) (0) (0) (1) (1) (2) (4)
G Tumour mutational burden in combination therapy group
Recurrence-free survival (%)
HR 0·74
(95% CI 0·48–1·16)
Time since randomisation (months)
Number at risk (number censored)
Low count 67 64 55 43 36 28 28 26 25 22 12 4 0 0
(0) (1) (1) (1) (1) (1) (1) (2) (2) (4) (13) (21) (24) (24)
High count 71 69 62 48 41 38 33 32 31 31 19 9 3 0
(0) (1) (3) (3) (3) (3) (4) (4) (5) (5) (15) (25) (31) (34)
H ctDNA in the tumour mutational burden subset in the combination therapy group
HR 2·50
(95% CI 1·44–4·34)
Time since randomisation (months)
Negative 118 113 103 83 71 62 57 55 53 50 29 13 3 0
(0) (2) (3) (3) (3) (3) (4) (5) (6) (8) (26) (42) (51) (54)
Positve 20 20 14 8 6 4 4 3 3 3 2 0 0 0
(0) (0) (1) (1) (1) (1) (1) (1) (1) (1) (2) (4) (4) (4)


High expression
Low expression
A IFNG in placebo group
Recurrence-free survival (%)
HR 0·67
(95% CI 0·46–0·99)
Number at risk
(number censored)
Low expression 85 49 37 32 27 26 24 22 22 22 16 10 4 0
(0) (2) (5) (5) (5) (5) (5) (6) (6) (6) (12) (18) (24) (28)
High expression 84 61 50 45 42 41 38 36 35 35 25 11 0 0
(0) (0) (0) (0) (1) (1) (2) (3) (3) (3) (12) (26) (37) (37)
Positive
Negative
B ctDNA in the IFNG subset in the placebo group
HR 3·55
(95% CI 2·24–5·63)
Negative 142 103 81 73 66 64 59 55 54 54 39 19 3 0
(0) (2) (5) (5) (6) (6) (7) (9) (9) (9) (23) (43) (59) (62)
Positve 27 7 6 4 3 3 3 3 3 3 2 2 1 0
(0) (0) (0) (0) (0) (0) (0) (0) (0) (0) (1) (1) (2) (3)
C IFNG in combination therapy group
Recurrence-free survival (%)
HR 0.49
(95% CI 0·31–0·75)
Number at risk
(number censored)
Low expression 89 84 75 61 49 42 40 39 36 33 16 8 1 0
(0) (0) (1) (1) (2) (2) (2) (2) (3) (5) (19) (27) (34) (35)
High expression 90 88 81 75 67 64 61 55 55 51 33 16 5 0
(0) (1) (2) (2) (3) (3) (4) (5) (5) (9) (25) (41) (52) (57)
D ctDNA in the IFNG subset in the combination therapy group
HR 2·47
(95% CI 1·41–4·33)
Negative 158 152 141 126 108 100 95 89 86 80 46 23 5 0
(0) (1) (2) (2) (4) (4) (5) (6) (7) (12) (41) (63) (81) (86)
Positve 21 20 15 10 8 6 6 5 5 4 3 1 1 0
(0) (0) (1) (1) (1) (1) (1) (1) (1) (2) (3) (5) (5) (6)

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