

Percorsi di Oncologia di Precisione: Appropriatezza diagnostica e Molecular Tumor Board

**30 GENNAIO 2026
MILANO**

INNSiDE by Meliá Milano Torre Galfa
Via Gustavo Fara, 41

**NGS, cura e ricerca: biomarcatori
per farmaci on-label e
sperimentazione clinica**

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Predictive/prognostic biomarker characterization in tumor and tumor microenvironment is paramount for therapy selection and drug benefit

- Gross (visual) examination for tumor stage and lymph node status
- Light microscopy for tumor histology (tumor type, grade, tumor infiltrating lymphocyte prevalence) and tumor stage/lymph node status confirmation
- Immunohistochemistry (HER2, PD-L1, Claudin 18.2)
- (Fluorescent) in situ hybridization – (F)ISH (HER2, ALK)
- RT-PCR (EGFR, KRAS, BRAF)
- Sanger sequencing
- Massive parallel sequencing («next» generation sequencing, NGS)

European Groundshot—addressing Europe’s cancer research challenges: a *Lancet Oncology Commission*



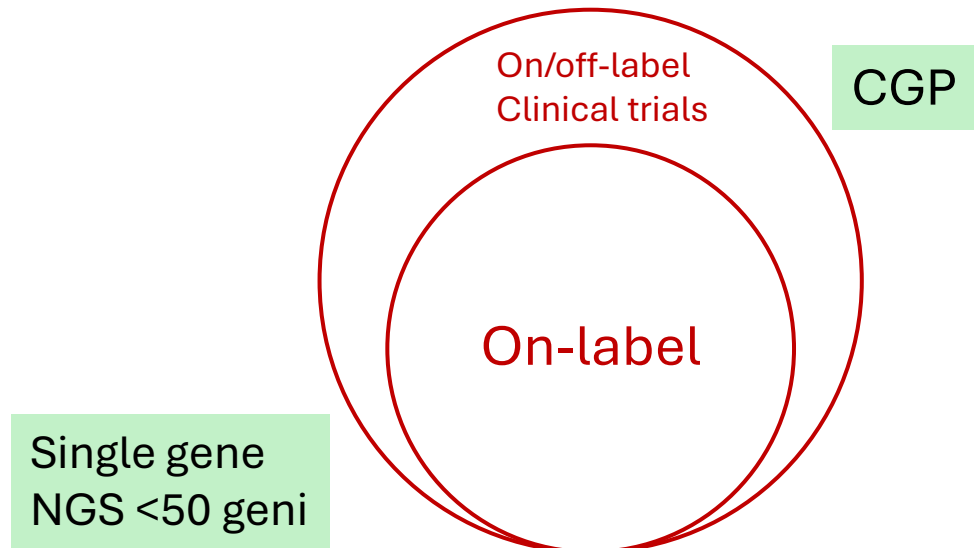
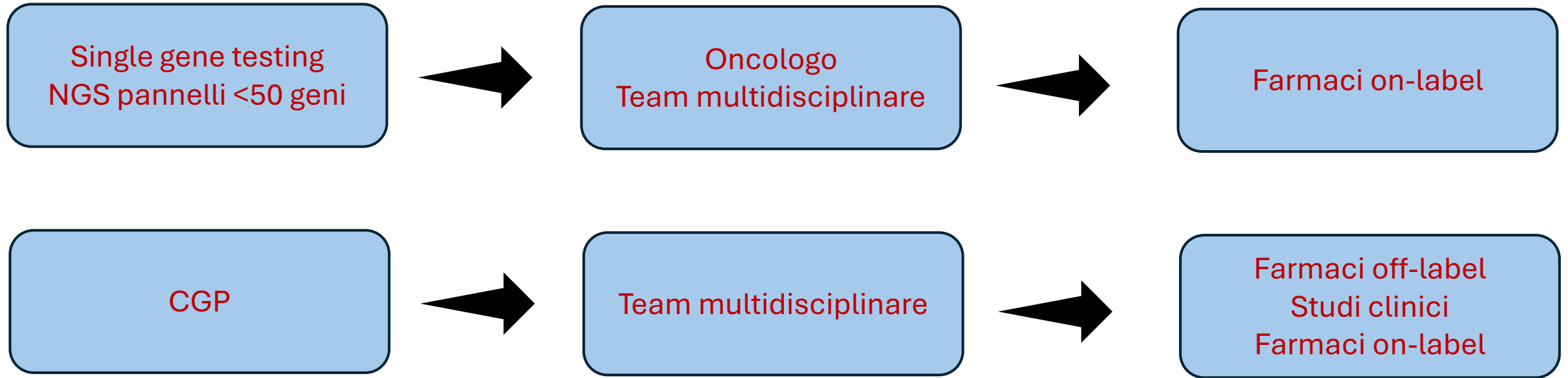
Mark Lawler, Lynne Davies, Simon Oberst, Kathy Oliver, Alexander Eggermont, Anna Schmutz, Carlo La Vecchia, Claudia Allemani, Yolande Lievens, Peter Naredi, Tanja Cufer, Ajay Aggarwal, Matti Aapro, Kathi Apostolidis, Anne-Marie Baird, Fatima Cardoso, Andreas Charalambous, Michel P Coleman, Alberto Costa, Mirjam Crul, Csaba L Dégi, Federica Di Nicolantonio, Sema Erdem, Marius Geanta, Jan Geissler, Jacek Jassem, Beata Jagielska, Bengt Jonsson, Daniel Kelly, Olaf Kelm, Teodora Kolarova, Tezer Kutluk, Grant Lewison, Françoise Meunier, Jana Pelouchova, Thierry Philip, Richard Price, Beate Rau, Isabel T Rubio, Peter Selby, Maja Južnič Sotlar, Gilliosa Spurrier-Bernard, Jolanda C van Hove, Eduard Vrdoljak, Willien Westerhuis, Urszula Wojciechowska, Richard Sullivan

A robust cancer biomarker infrastructure must be embedded across health systems, to ensure their deployment as innovation drivers across Europe

Embedding cancer biomarkers within real-world oncology delivery and providing genomic testing across Europe, while ensuring that inequity gaps for patients are narrowed and not widened, must be the goal

If deployed appropriately, cancer biomarkers can reduce costs by ensuring the right treatment, for the right patient, at the right dose, at the right time. Using cancer biomarkers can avoid specific cancer treatment sequelae for patients who gain no therapeutic benefit from these treatments

Oncologia di precisione, due processi distinti



The case for NGS over single-gene testing (IHC/ISH/FISH)

Guidelines

ESMO 2020

Next-generation sequencing (NGS) allows sequencing of a high number of nucleotides in a short time frame at an affordable cost. While this technology has been widely implemented, there are no recommendations from scientific societies about its use in oncology practice. The European Society for Medical Oncology (ESMO) is proposing three levels of recommendations for the use of NGS. Based on the current evidence, ESMO recommends routine use of NGS on tumour samples in advanced non-squamous non-small-cell lung cancer (NSCLC), prostate cancers, ovarian cancers and cholangiocarcinoma. In these tumours, large multigene panels could be used if they add acceptable extra cost compared with small panels. In colon cancers, NGS could be an alternative to PCR. In addition, based on the KN158 trial and considering that patients with endometrial and small-cell lung cancers should have broad access to anti-programmed cell death 1 (anti-PD1) antibodies, it is recommended to test tumour mutational burden (TMB) in cervical cancers, well- and moderately-differentiated neuroendocrine tumours, salivary cancers, thyroid cancers and vulvar cancers, as TMB-high predicted response to pembrolizumab in these cancers. Outside the indications of multigene panels, and considering that the use of large panels of genes could lead to few clinically meaningful responders, ESMO acknowledges that a patient and a doctor could decide together to order a large panel of genes, pending no extra cost for the public health care system and if the patient is informed about the low likelihood of benefit. ESMO recommends that the use of off-label drugs matched to genomics is done only if an access programme and a procedure of decision has been developed at the national or regional level. Finally, ESMO recommends that clinical research centres develop multigene sequencing as a tool to screen patients eligible for clinical trials and to accelerate drug development, and prospectively capture the data that could further inform how to optimise the use of this technology.

Key words: next-generation sequencing (NGS), genomic alterations, metastatic cancers

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: A report from the ESMO Precision Medicine Working Group. Ann Oncol. 2020.

ESMO 2024

Background: Advancements in the field of precision medicine have prompted the European Society for Medical Oncology (ESMO) Precision Medicine Working Group to update the recommendations for the use of tumour next-generation sequencing (NGS) for patients with advanced cancers in routine practice.

Methods: The group discussed the clinical impact of tumour NGS in guiding treatment decision using the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) considering cost-effectiveness and accessibility.

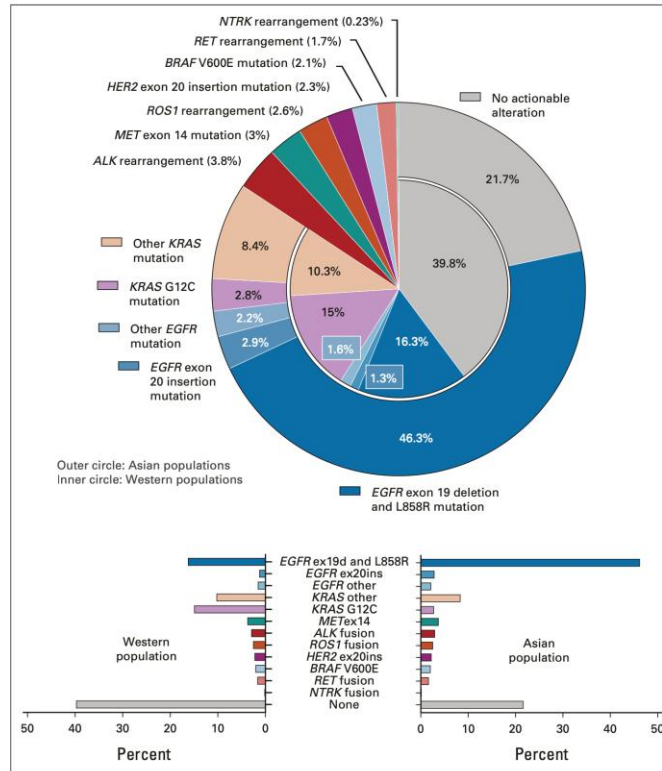
Results: As for 2020 recommendations, ESMO recommends running tumour NGS in advanced non-squamous non-small-cell lung cancer, prostate cancer, colorectal cancer, cholangiocarcinoma, and ovarian cancer. Moreover, it is recommended to carry out tumour NGS in clinical research centres and under specific circumstances discussed with patients. In this updated report, the consensus within the group has led to an expansion of the recommendations to encompass patients with advanced breast cancer and rare tumours such as gastrointestinal stromal tumours, sarcoma, thyroid cancer, and cancer of unknown primary. Finally, ESMO recommends carrying out tumour NGS to detect tumour-agnostic alterations in patients with metastatic cancers where access to matched therapies is available.

Conclusion: Tumour NGS is increasingly expanding its scope and application within oncology with the aim of enhancing the efficacy of precision medicine for patients with cancer.

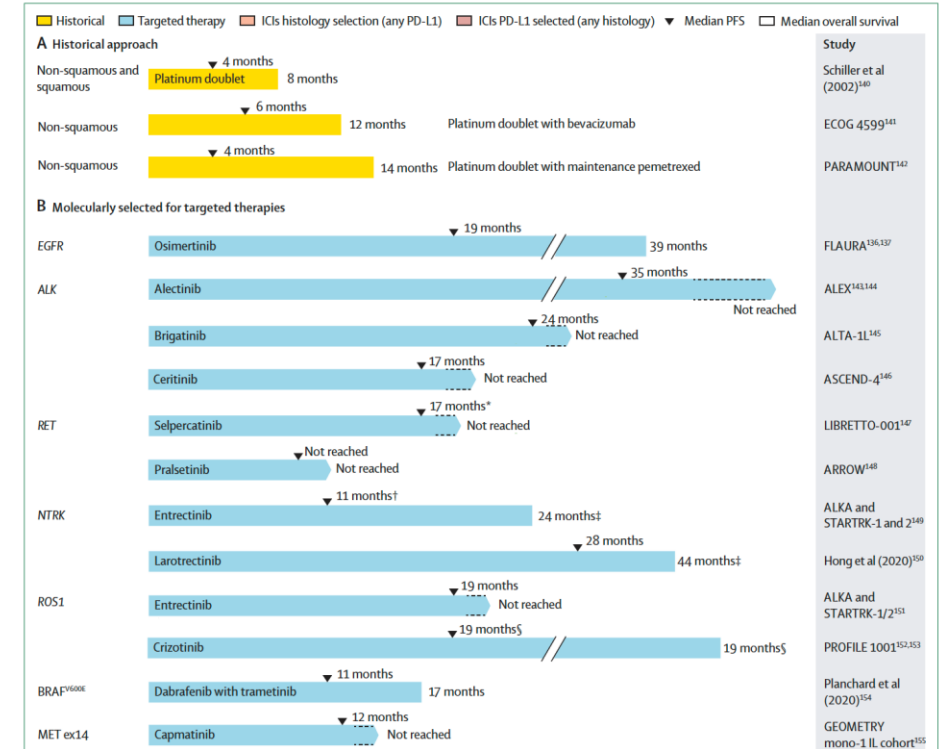
Key words: next-generation sequencing (NGS), advanced cancer, precision medicine, ESCAT

Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2024.

ESCAT scale IA genomic variants and personalized therapy in NSCLC patients



Tan&Tan, 2024



Thai, Lancet, 2021

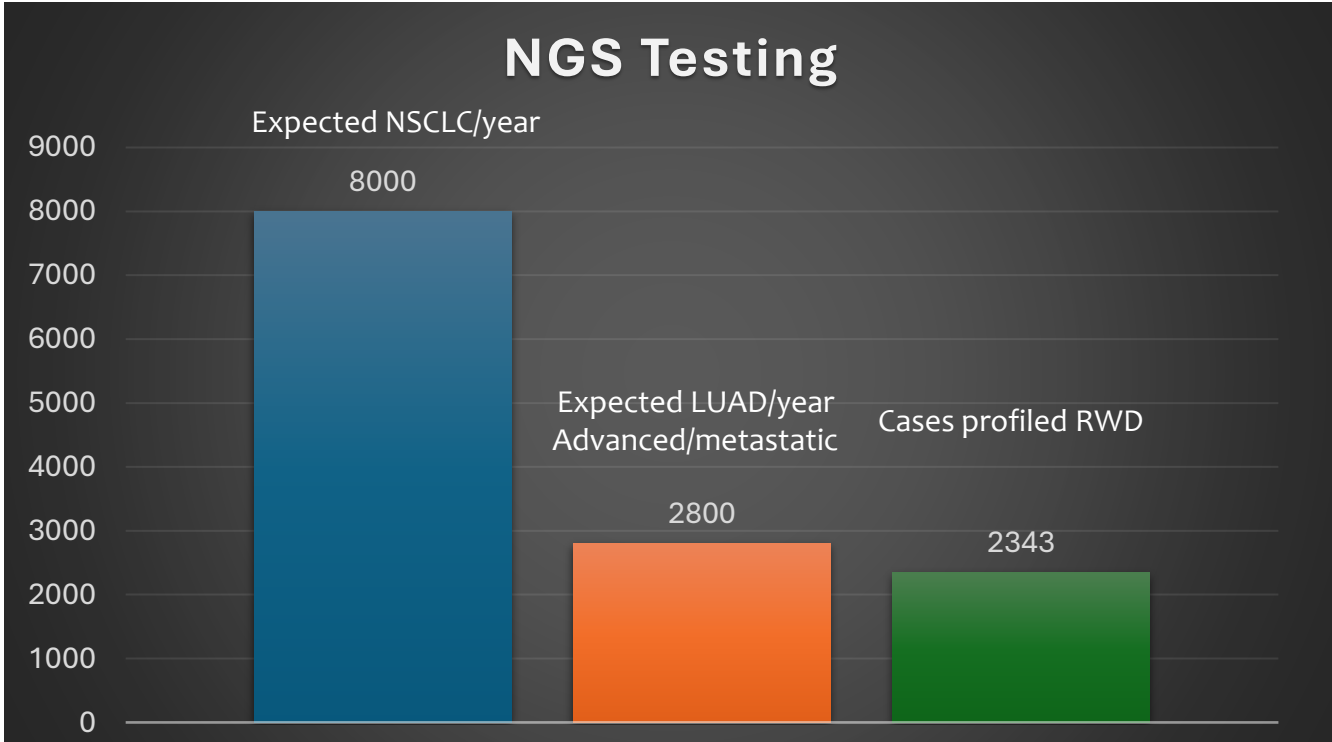
Italy - Ministry of Health decree 2022

ESCAT IA Biomarkers for NSCLC patient therapy

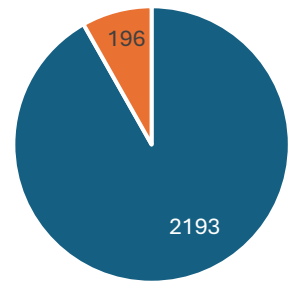
- KRAS G12C
- EGFR
- BRAF
- ALK
- ROS1
- NTRK
- RET
- MET exon skipping
- HER2

Struttura Sanitaria	Laboratorio	Azzonamento Indicativo
Fondazione IRCCS Istituto Nazionale dei Tumori	Anatomia Patologica 2	Pazienti in carico alla Struttura e alle altre Strutture della ATS di Milano Città Metropolitana e della ATS di Bergamo
ASST Santi Paolo e Carlo	Anatomia Patologica	Pazienti in carico alla Struttura e alle altre Strutture della ATS Città Metropolitana di Milano
Fondazione IRCCS Ca' Granda – Ospedale Maggiore Policlinico	Anatomia Patologica	Pazienti in carico alla Struttura e alle altre Strutture della ATS Città Metropolitana di Milano
ASST Sette Laghi	Anatomia Patologica	Pazienti in carico alla ASST Sette Laghi e alle altre Strutture della ATS dell'Insubria e della ATS della Montagna
Fondazione IRCCS Policlinico San Matteo	Anatomia Patologica	Pazienti in carico alla IRCCS San Matteo Pavia e alle altre Strutture della ATS di Pavia e della ATS della Val Padana
ASST Grande Ospedale Metropolitano Niguarda	Anatomia Patologica	Pazienti in carico alla Struttura e alle altre Strutture della ATS di Città Metropolitana Milano e della ATS della Brianza
ASST degli Spedali Civili di Brescia	Anatomia Patologica	Pazienti in carico alla Struttura e alle altre Strutture della ATS di Brescia e della ATS della Montagna
IRCCS Ospedale San Raffaele	Anatomia Patologica	Pazienti in carico alla Struttura
IRCCS Humanitas Mirasole Reserch Hospital	Anatomia Patologica	Pazienti in carico alla Struttura
IRCCS Istituto Europeo di Oncologia, Milano	Dipartimento di Anatomia Patologica e Medicina di Laboratorio	Pazienti in carico alla Struttura

Lung cancer- NGS Testing Regione Lombardia 2023

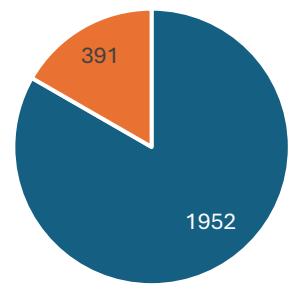


Clinical setting



■ First line ■ Progression to target therapy

Type of panel

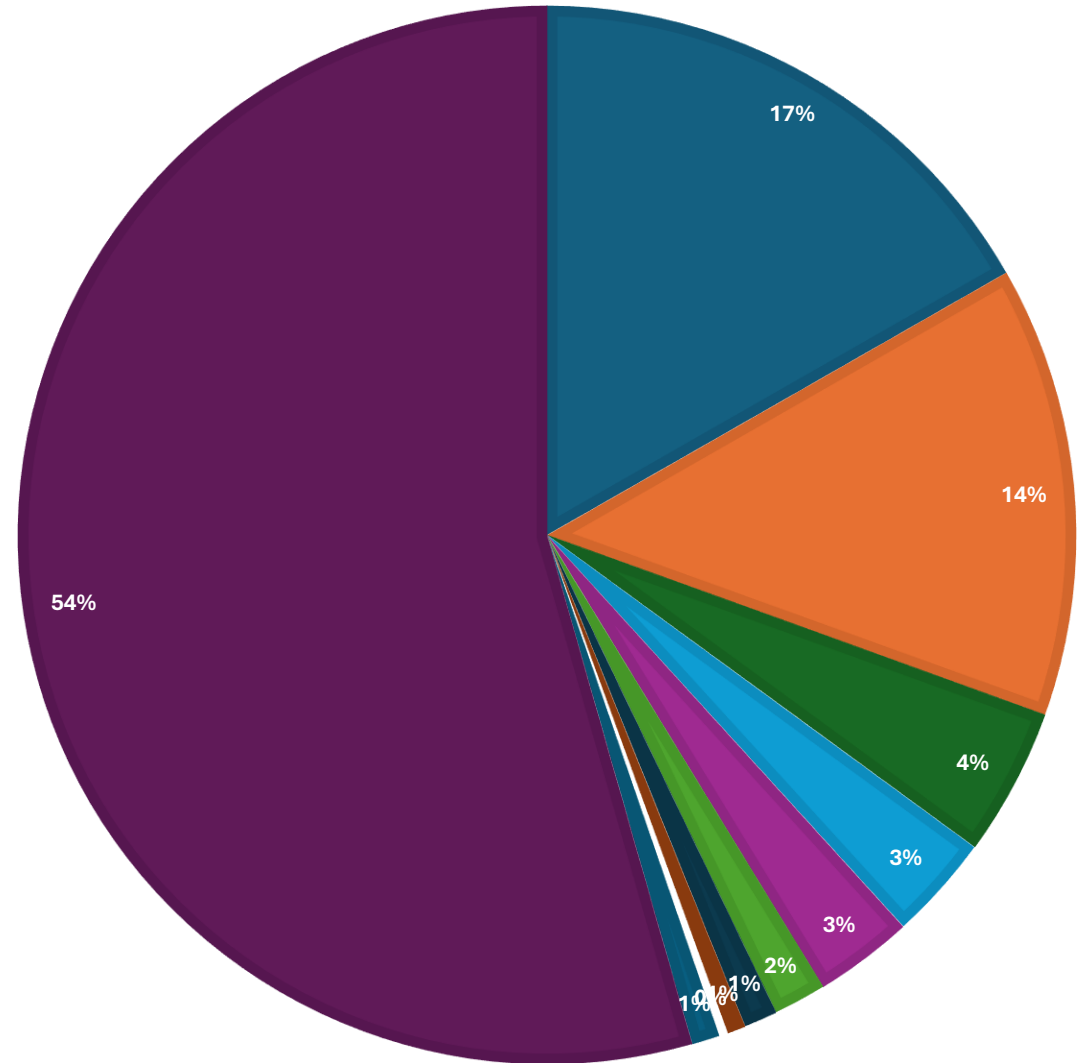


■ Targeted ■ Comprehensive

Variants

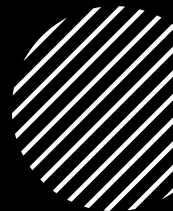
Molecular Alteration	N. Cases
EGFR mutation	400
KRAS p.Gly12Cys	333
ALK rearrangement	110
BRAF p.Val600Glu	80
MET exn 14 skipping	72
ERBB2 mutation	43
RET rearrangement	27
ROS1 rearrangement	14
NTRK1-3 rearrangement	6
Multiple genetic alterations	20

■ EGFR ■ KRAS p.G12C ■ ALK ■ BRAF ■ MET ■ ERBB2 ■ RET ■ ROS1 ■ NTRK1-3 ■ Multiple ■ No druggable alteration





Why would we need extensive profiling?



Potentially useful biomarkers for patient care (phase I/II trials)



Mutational profiles associated with therapy resistance (immunotherapy e TKIs)



Potential germline variants (BRCA)

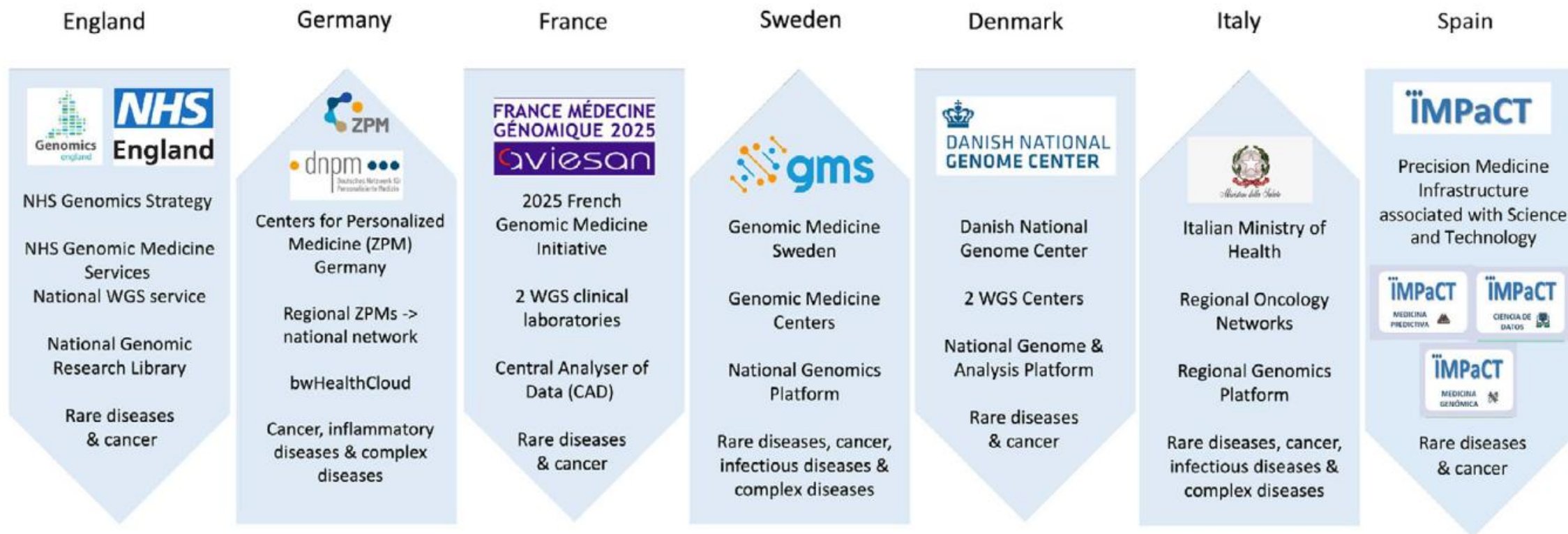


Agnostic biomarkers (NTRK, RAS)



Complex biomarkers (mutational tumor burden, homologous recombination deficiency), CNV

Precision medicine networks in Europe



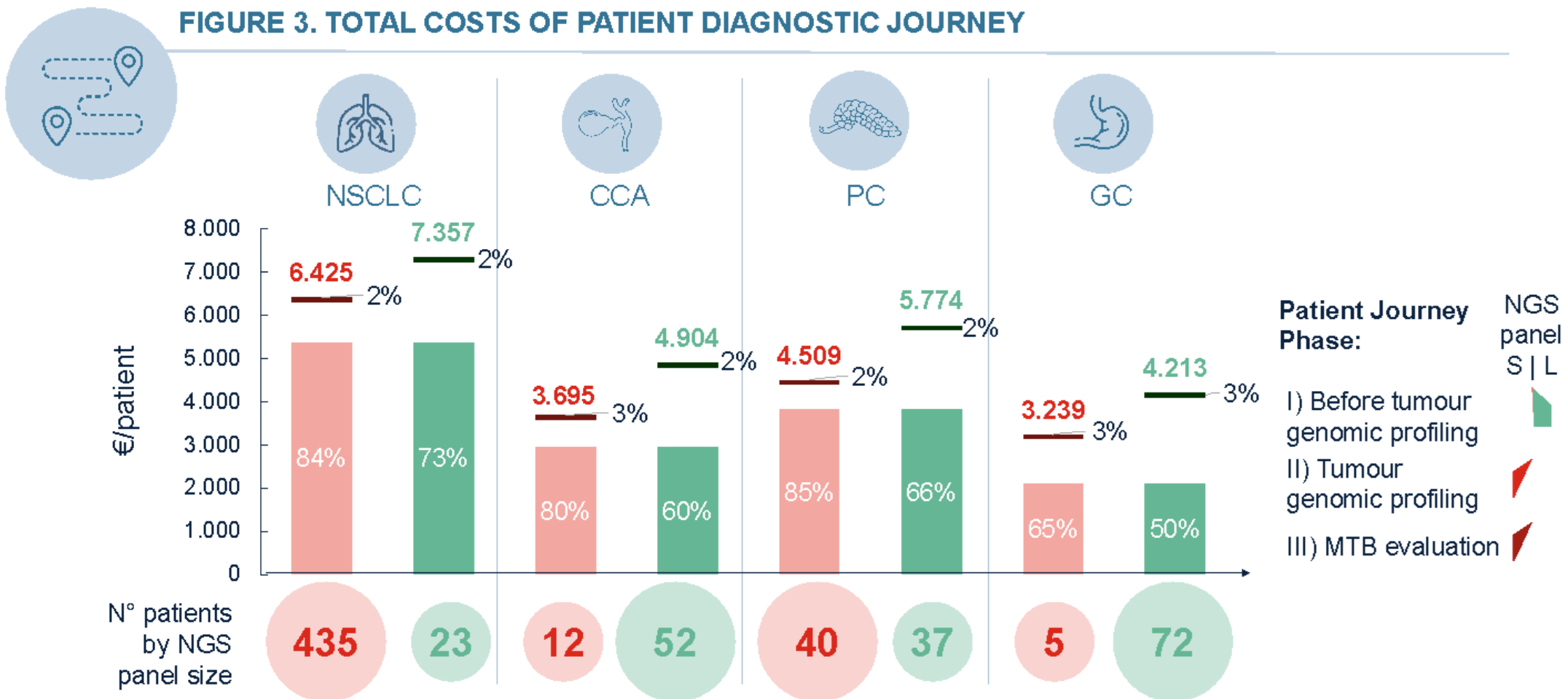
Targeted CGP is the most cost effective approach

Table 1. NGS technologies				
	Targeted NGS	WES	RNAseq	WGS
Coverage	Specific regions	Exonic regions of all protein-coding genes		Exonic and intronic regions
Size	1-2.0 Mb	35-60 Mb		3.4 Gb
Depth	500-20,000X	T: 150-200X N: 100X	Depending on the quantity of data generated	T: 60-80X N: 30-40X
Tumor purity	Less stringent	Stringent	Stringent	Stringent
Sensitive to the sample quality	+	++	++	+++
Turnaround time	Days	Weeks	Weeks	Weeks
Costs	++	+++	++	++++
Tested parameters	TMB+ MSI+ (Mutational sig) HRD+ SNV quality+++ Driver SNV++ SCNA+ (Fusions/Rearrangements)+	TMB++ MSI+++ Mutational sig++ HRD+++ SNV quality+++ Driver SNV++ SCNA++ (Fusions/Rearrangements)+	SNV quality+ Driver SNV+ Fusion+++ Expression+++ Exon skipping+++	TMB+++ MSI+++ Mutational sig+++ HRD+++ SNV quality+++ Driver SNV++ SCNA+++ Fusions/Rearrangements++

HRD, homologous repair deficiency; Mb, Megabase; MSI, microsatellite instability; N, normal; NGS, next-generation sequencing; SCNA, somatic copy number alteration; SNV, single nucleotide variant; T, tumor; TMB, tumor mutational burden; WES, whole-exome sequencing; WGS, whole-genome sequencing.

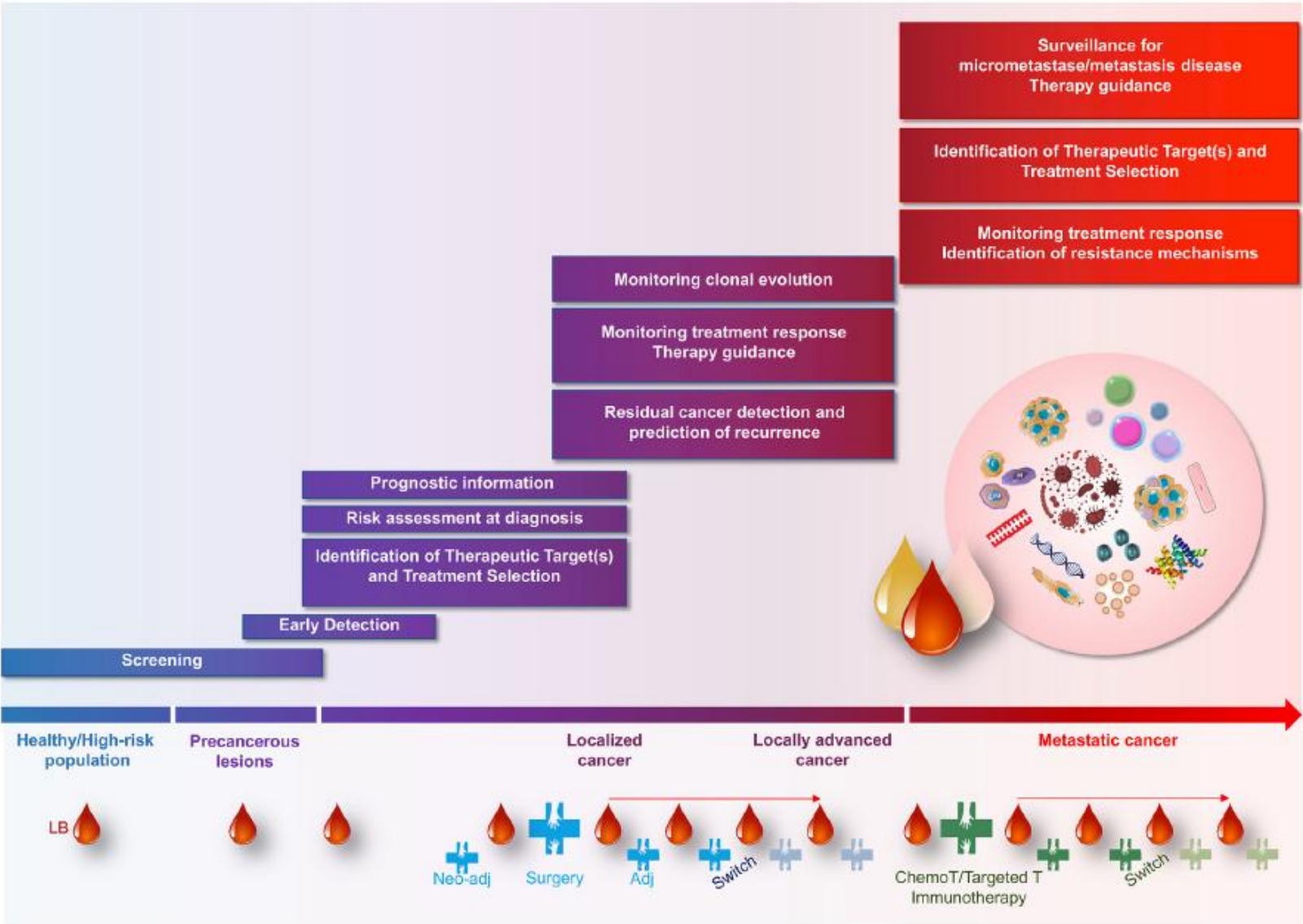
Il costo dei pannelli NON impatta significativamente nel percorso diagnostico

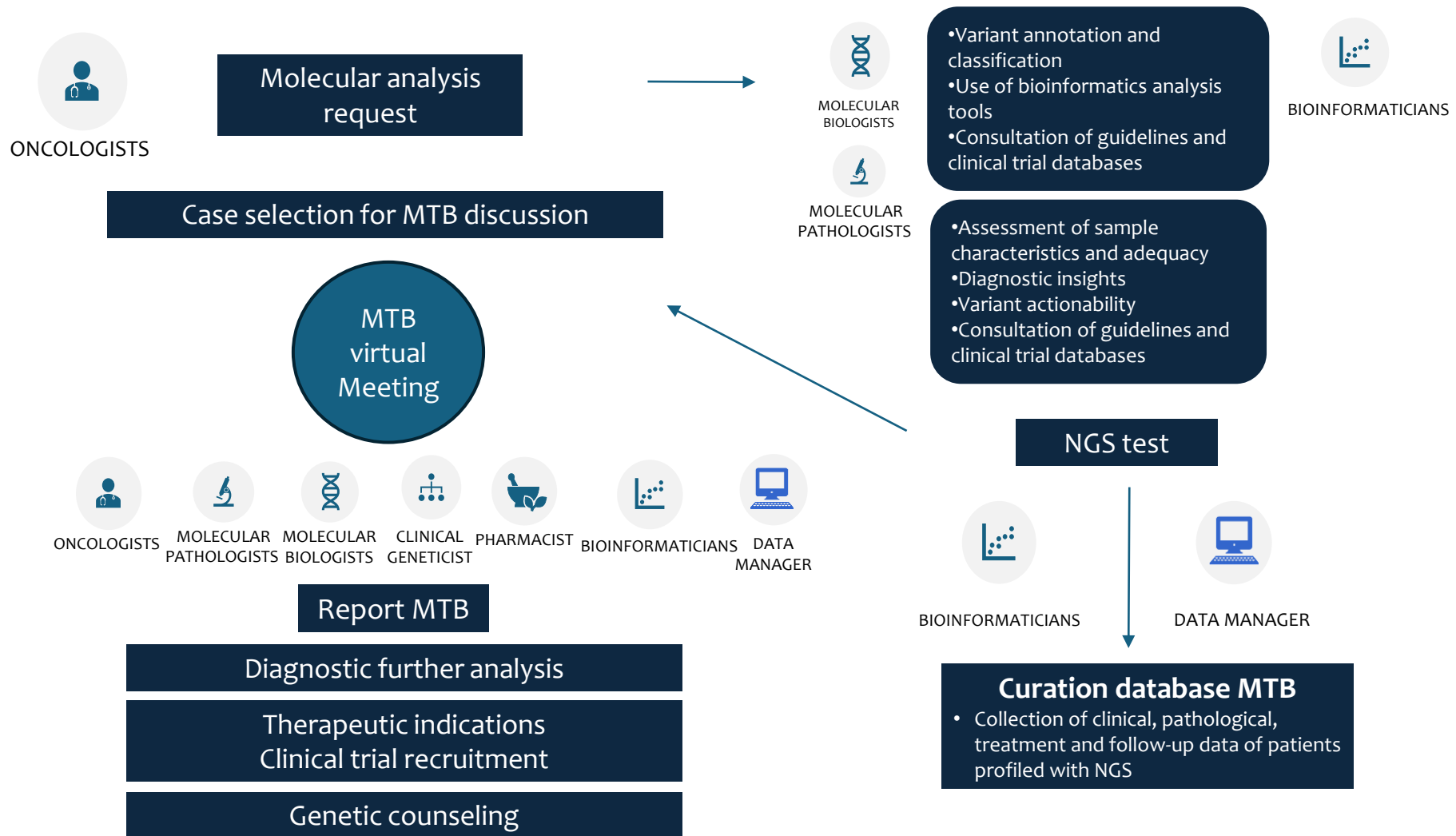
FIGURE 3. TOTAL COSTS OF PATIENT DIAGNOSTIC JOURNEY



Current landscape of clinically used LB assays

Indications/clinical settings needing LB assays/platforms





Chairs: DeBraud/Pruneri
 Coordinators: Vingiani/Duca

Demographics
Clinical record

Anamnestic data

Pathological diagnosis

Mutational profile

TMB value

Therapeutic indication

Genetic counseling

NGS panel data

Discussione Collegiale Molecular Tumor Board

Quesito diagnostico: Valutazione Terapeutica paziente non presente

Anamnesi Patologica Prossima: A seguito di comparsa di DM misto ID a Giugno 2021, con perdita di peso > 10 kg, eseguita:
24/07/21 Ecografia addome: formazione di 43 in S6; in corrispondenza dell'ilo renale sx formazione esofittica di 23 mm
03/08/2021 RMN: al VI segmento formazione di 35 mm possibile alterazione vascolare. In sede sovrenale sx formazione di 49 mm
03/08/2021 metanefrina U 276 (< 320), normetanefrina U 3807 (< 390), aldosterone 45.6 (< 21.4), renina 93.9 (46.1), CgA 856 (< 100), NSE, ACTH e cortisolo nei limiti.
29/09/2021 Scintigrafia MIBG: Si osserva un accumulo patologico del tracciante sola della formazione più piccola segnalata alla RM in corrispondenza del braccio mediale del surrene sinistro.
04/10/2021 Valutazione urologica specialistica (dott. Nicolai): Il quadro assume le caratteristiche di paraganglioma paraortico sottorenale e massa surrenalica coerente con adenoma non secernente.
14/12/2021 Exeresi paraganglioma sinistro videolaparoscopico e surrenectomia sinistra. Et: A) *Surrene sinistro: feocromocitoma del surrene, limitato alla ghiandola. Positività immunocitochimica per sinaptofisina, SF1, S100 (cellule sustentacolari). Mantenuta espressione immunocitochimica di SDHB. Ki-67>3%: assente. PASS SCORE: >= 4 (sec AFIP serie 4/2007). B) Paraganglioma con tessuto para-aortico sinistro: metastasi linfonodale di feocromocitoma.*

Diagnosi: Feocromocitoma surrenalico con metastasi linfonodale operato

Esito della discussione:
L'esito delle analisi molecolari riportate nel referto T22-204 eseguite mediante sequenziamento di nuova generazione è stato oggetto di discussione multidisciplinare nel contesto del Molecular Tumor Board istituzionale, in data 2022-03-17. Le alterazioni molecolari sono state valutate e classificate secondo le linee guida AMP-ASCO-CAP (Li MM et al., J Mol Diagn. 2017 Jan;19(1):4-2), con il seguente esito:

Gene	Variante cDNA	Variante aminoacidica	Classificazione	Frequenza allelica
DDX4	c.827T>C	p.Val276Ala (V276A)	VUS	52%
MAX	c.196A>T	p.Lys66Ter (K66*)	Pathogenic	63%
BLM	c.2784T>G	p.Asp928Glu (D928E)	VUS	51%
ADAT1	c.505T>G	p.Ser169Ala (S169A)	VUS	56%
RAD51D	c.775C>T	p.Arg259Trp (R649W)	VUS	39%
RET	c.1946C>T	p.Ser649Leu (S649L)	Risultati discordanti	51%
FANCF	c.1_2delAT		VUS	25%
wt	NA			

Il valore del TMB è 2.85 muts/Mbp.

In considerazione della presenza della variante p.S649L del gene RET, per la quale sono presenti in letteratura ed in database genomiche evidenze a favore di potenziale patogenicità, si segnala possibile sensibilità a Selpercatinib.
In assenza di valide alternative terapeutiche, si segnala disponibilità di protocollo INT 63/18, in fase di arruolamento presso il nostro Istituto.

In considerazione della variante K66* del gene MAX e della variante S649L del gene RET si ritiene indicata consulenza genetica.

Metodica di analisi NGS.Pannello Oncomine Comprehensive Assay Plus (DNA) mediante tecnologia IonTorrent (Thermo Fisher Scientific, Life Technologies), per l'analisi delle alterazioni molecolari dei seguenti geni:

A1CF, ABCB1, ABL1, ABL2, ABRAXAS1, ACS2M2B, ACVR1B, ACVR2A, ADAM10, ADAMTS12, ADAMTS2, AKT1, AKT2, AKT3, ALK, AMER1, ANO4, APC, AR, ARAF, ARHGAP35, ARID1A, ARID1B, ARID2, ARID5B, ARMC4, ASXL1, ASXL2, ATM, ATRP1A1, ATR, ATRX, AURKA, AURKB, AURKC, AXIN1, AXIN2, AXL, B2M, BAP1, BARD1, BCL2, BCL2L12, BCL6, BCOR, BCR,

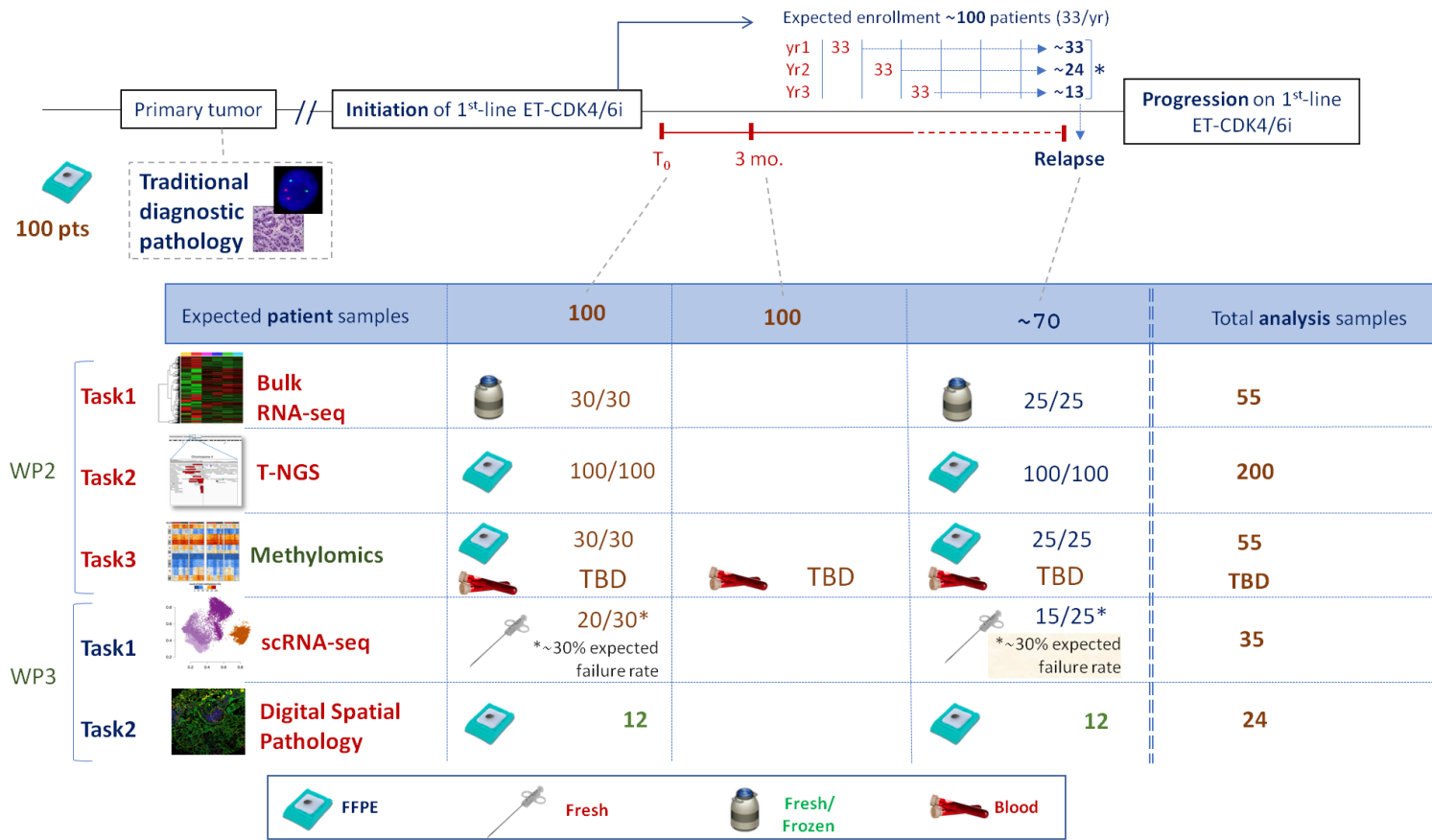
Il presente referto è una rappresentazione, su supporto cartaceo, del documento elettronico firmato digitalmente ai sensi della normativa vigente rinvenibile presso la Fc
Firmatario: Duca Matteo - Data e ora de

Il tuo 5 per mille per finanziare la ricerca
"Finanziamento della ricerca sanitaria
e nella cura dei tumori"

3-400 report/year

UNDERSTAND

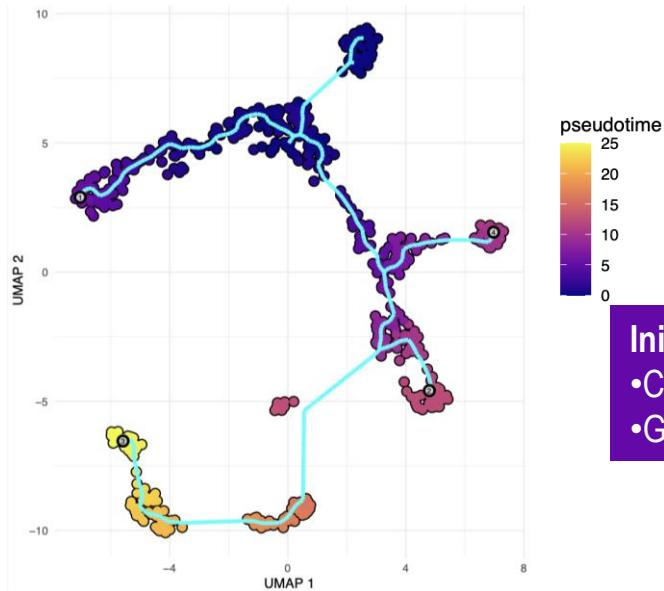
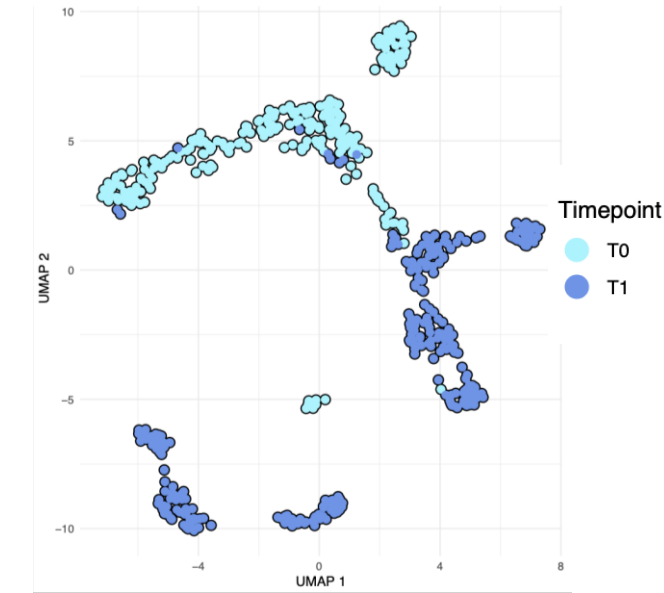
Unraveling tumor resistance mechanisms in HR+ aDvancEd bReaST cANcer undergoing CDK4/6 inhibitors therapy



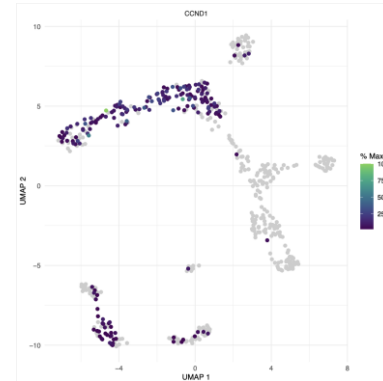
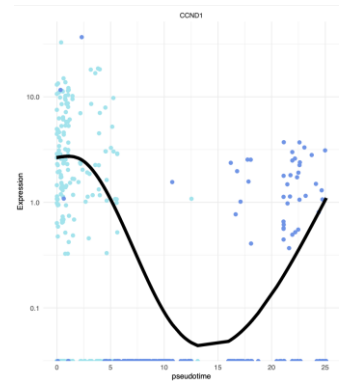
The UNDERSTAND study investigates the mechanisms of primary and acquired resistance to CDK4/6 inhibitors in advanced HR+/HER2- breast cancer, utilizing multi-omic analyses on longitudinally collected tumor samples

Characteristics	Overall Cohort	Cohort A	Cohort B
Patients	73	32	41
Median age (IQR)	69 (52-75)	62 (52-72)	69 (52-79)
Ethnicity			
White	71 (97.3%)	30 (93.7%)	41 (100)
Black	2 (2.7%)	2 (6.3%)	0
Menopausal status (%)			
Pre	15 (20.5%)	6 (18.7%)	9 (17%)
Post	58 (79.5%)	26 (81.3%)	32 (83%)
Histologic Grade (%)			
G1	4 (5.5%)	0	2 (4.9%)
G2	52 (71.2%)	21 (65.6%)	32 (78%)
G3	17 (23.3%)	11 (34.4%)	7 (17.1%)
Metastatic Sites n° (%)			
1	37 (50.7%)	13 (58.5%)	24 (58.5%)
2	31 (42.5%)	16 (50%)	15 (36.6%)
≥3	5 (6.8%)	3 (9.4%)	2 (4.9%)
Site of metastases n° (%)			
Breast	12 (16.4%)	9 (28.1%)	3 (7.3%)
Bone	55 (75.3%)	14 (43.7%)	41 (100%)
Visceral	13 (17.8%)	8 (25%)	5 (12.2%)
Lymphnode	16 (21.9%)	12 (37.5%)	4 (9.7%)
Other	7 (9.6%)	4 (12.5%)	3 (7.3%)
Adjuvant Chemotherapy (%)			
CT	25 (34.2%)	10 (31.2%)	6 (14.6%)
No CT	48 (65.8%)	22 (68.8%)	35 (85.4%)
Adjuvant Hormone Therapy (%)			
Tam	19 (26%)	6 (18.7%)	16 (39%)
Tam+ LHRH	17 (23.3%)	9 (28.1%)	9 (21.9%)
AI	11 (15.1%)	2 (6.2%)	7 (17.1%)
No HT	26 (35.6%)	15 (48.9%)	9 (22%)
First line Therapy (%)			
Abema + AI	23 (31.5%)	9 (28.1%)	14 (34.1%)
Ribo + AI	30 (41%)	16 (50%)	14 (34.1%)
Palbo + AI	3 (4.1%)	0	3 (7.3%)
Ribo + Fulv	1 (1.4%)	1 (3.1%)	0
Abema + Fulv	16 (21.9%)	6 (18.7%)	10 (24.3%)
Follow-up			
Median FU time (IQR)	13.3 (4.3-20.3)	5.9 (2.2-14.6)	18.1 (8.0-22.5)
PD rate	21.9%	18.2%	25%

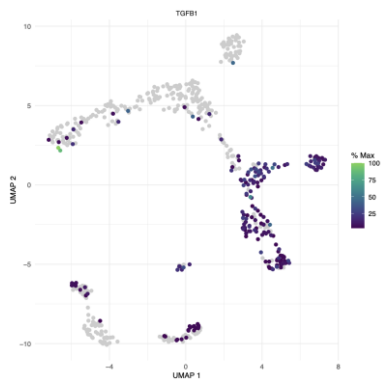
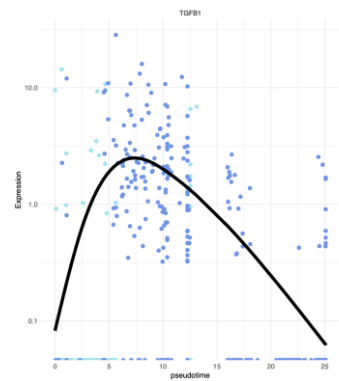
Longitudinal evolution



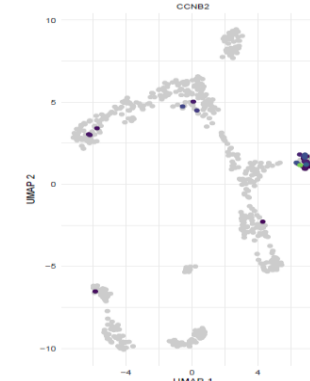
CCND1



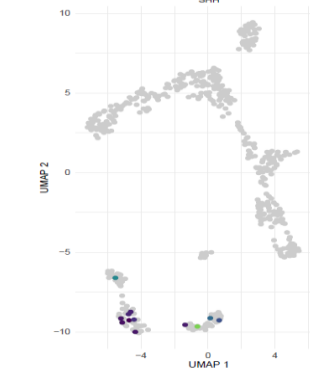
TGFB2



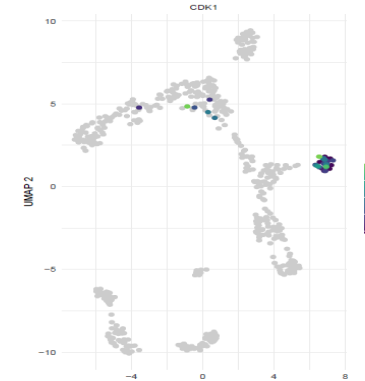
CCNB1



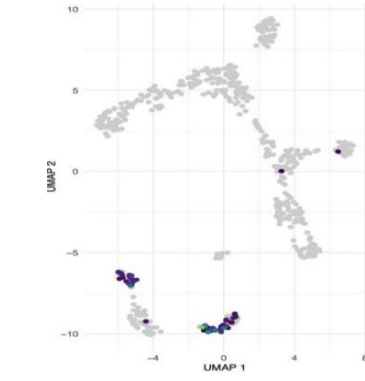
SHH



CDK1



CYP3A4



Initial Response

- CCND1 suppression
- G1/S arrest

Bypass Activation

- CCNB1/CDK1 upregulation
- Mitotic escape

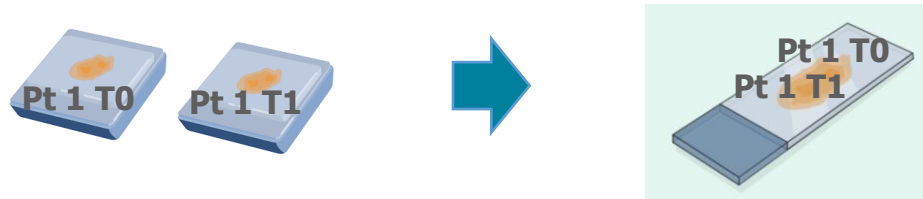
Resistance Programs

- Hedgehog pathway
- Drug metabolism

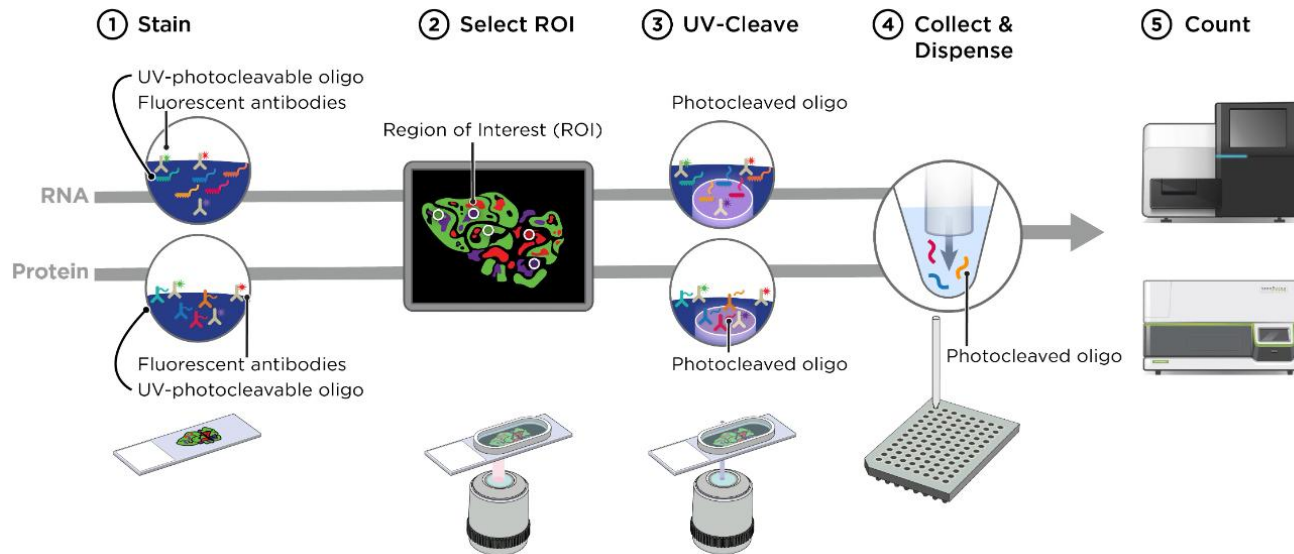
UNDERSTAND

Unraveling tumor resistance mechanisms in HR+ aDvancEd bReaST cANcer undergoing CDK4/6 inhibitors therapy

Task 4: Spatial Transcriptomics– Nanostring GeoMX WTA Panel >18000 genes



Ongoing...



Takeaways



A structured, sustainable and interconnected (pathology) molecular lab is vital in precision oncology



NGS with small panels is the backbone of cancer patient care



Targeted CGP identifies biomarkers for experimental approaches in the context of regional/national networks



The structure of reference labs promotes innovation (WES, WGS, scRNAseq, spatial transcriptomic)



Costs are not the (unique) issue, what really counts is organization