Methylomic profiling of melanoma tissue under anti-PD-1 immunotherapy: Identification of resistance mechanisms

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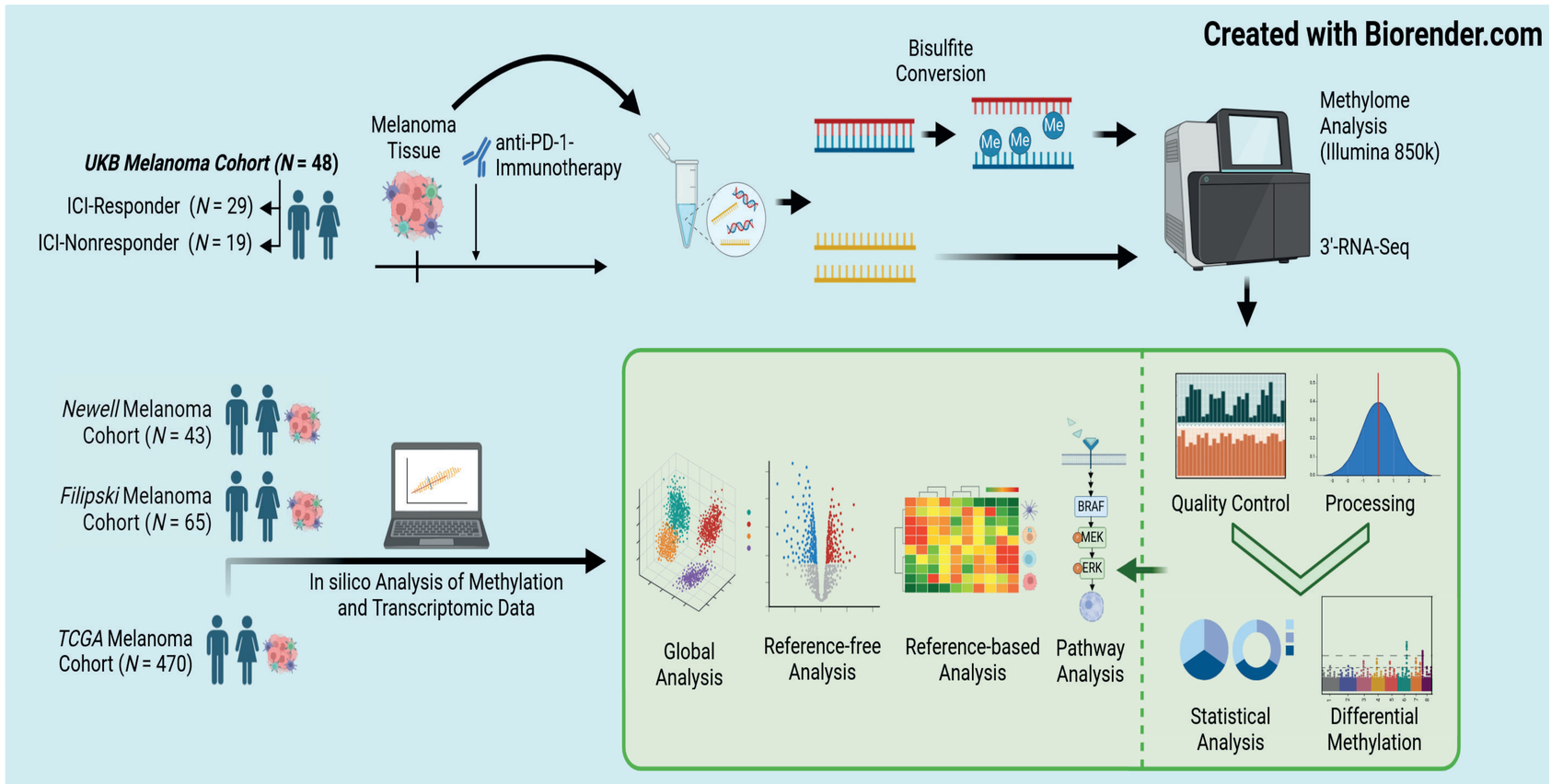


Figure 1: Approach.

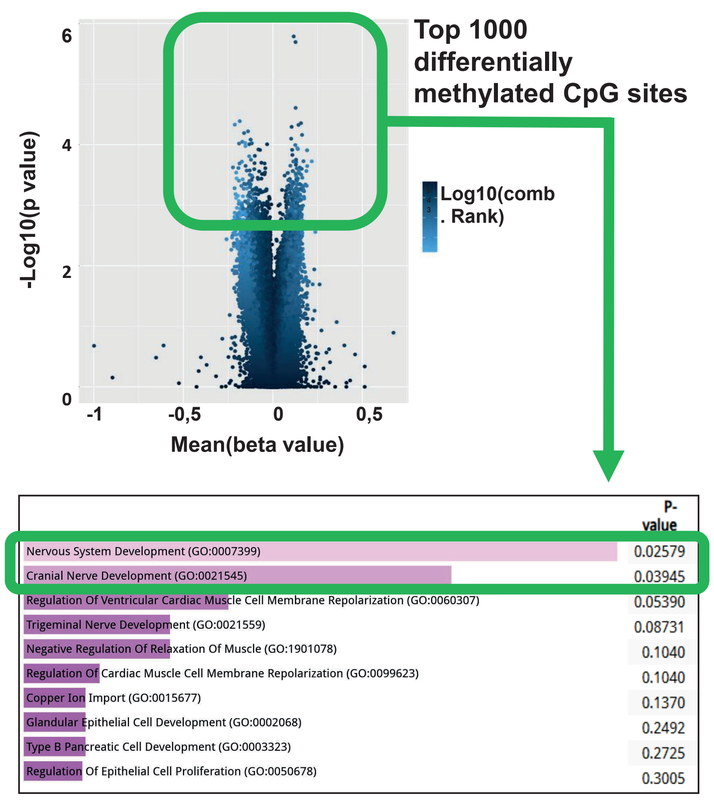
Clinical Characteristics

	All Patients (N=48)	Disease Control (N=29)	Progressive Disease (N=19)
Sex			
Female	15 (31.2%)	8 (27.6%)	7 (36.8%)
Male	33 (68.8%)	21 (72.4%)	12 (63.2%)
BRAF Mutation			
No	35 (72.9%)	21 (72.4%)	14 (73.7%)
Yes	13 (27.1%)	8 (27.6%)	5 (26.3%)
NRAS Mutation			
No	25 (52.1%)	14 (48.3%)	11 (57.9%)
Yes	22 (45.8%)	14 (48.3%)	8 (42.1%)
Not available	1 (2.1%)	1 (3.4%)	0 (0%)
Tissue Type			
Distant Metastasis	11 (22.9%)	7 (24.1%)	4 (21.1%)
Lymph Node	11 (22.9%)	4 (13.8%)	7 (36.8%)
Primary Tumor	7 (14.6%)	6 (20.7%)	1 (5.3%)
Skin	19 (39.6%)	12 (41.4%)	7 (36.8%)
Response			
complete response	17 (35.4%)	17 (58.6%)	0 (0%)
partial response	11 (22.9%)	11 (37.9%)	0 (0%)
stable disease	1 (2.1%)	1 (3.4%)	0 (0%)
progressive disease	19 (39.6%)	0 (0%)	19 (100%)
Age (Years)			
	72 (52 - 78)	72 (55 - 79)	71 (50 - 78)
OS (Months)			
	50 (9 - 79)	71 (47 - 85)	8 (5 - 13)
PFS (Months)			
	16 (3 - 66)	48 (34 - 80)	3 (2 - 4)

Figure 2: Clinical characteristics of the UKBonn cohort.

Introduction:

Most patients with metastatic melanoma develop primary or secondary resistance to therapy, reducing the effectiveness of systemic treatments. In this context, epigenetic regulatory mechanisms can drive dedifferentiation and contribute to tumor plasticity. This project aims to uncover epigenetically regulated patterns, genes, and signaling pathways involved in (de)differentiation, plasticity, and immune cell interactions in melanoma to identify potential biomarker candidates and clinically relevant targets to overcome therapy resistance.

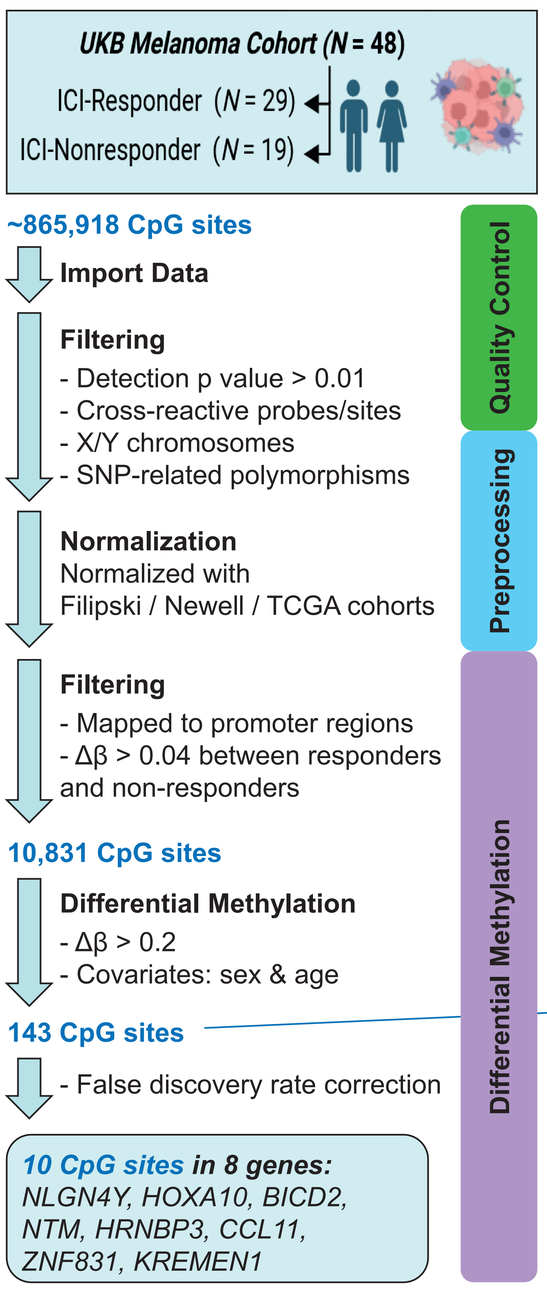


	P-value
Nervous System Development (GO:0007399)	0.02579
Cranial Nerve Development (GO:0021545)	0.03945
Regulation Of Ventricular Cardiac Muscle Cell Membrane Repolarization (GO:0060307)	0.05390
Trigeminal Nerve Development (GO:0021559)	0.08731
Negative Regulation Of Relaxation Of Muscle (GO:1901078)	0.1040
Regulation Of Cardiac Muscle Cell Membrane Repolarization (GO:0099623)	0.1040
Copper Ion Import (GO:0015677)	0.1370
Glandular Epithelial Cell Development (GO:0002068)	0.2492
Type B Pancreatic Cell Development (GO:0003323)	0.2725
Regulation Of Epithelial Cell Proliferation (GO:0050678)	0.3005

Figure 3: Enrichment analysis of top 1000 differentially methylated genes between responders and non-responders reveals association with pathways of the nervous system.

Materials & Methods:

Tumor samples from *N* = 48 patients with metastatic melanoma collected prior to anti-PD-1 immunotherapy were stratified into clear responders and non-responders, forming a case-control-like study cohort (**Figure 1**). Genome-wide DNA methylation and transcriptome profiling were performed using the Illumina EPIC (850k) array and 3′-RNA sequencing.

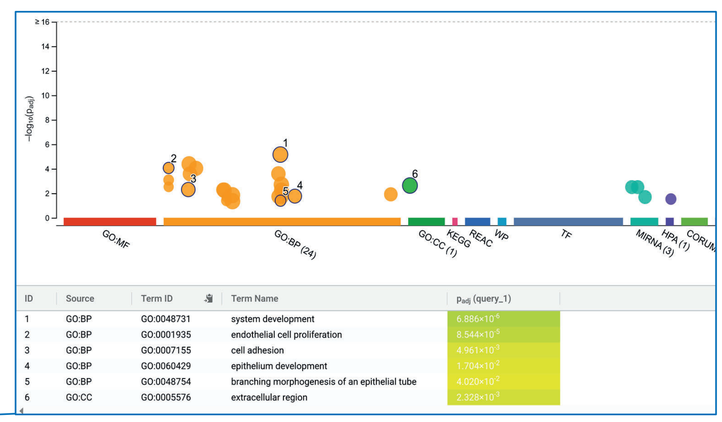


Results:

The UKBonn cohort was characterized by mutational status, tissue origin, survival, and therapy response (**Figure 2**). Differential methylation analysis between responders and non-responders identified distinct CpG sites. The top 1,000 were used for gene annotation and enrichment analysis (**Figure 3**), revealing pathways related to neural differentiation. Basic methylation analysis, including quality control, preprocessing, filtering, and differential methylation resulted in 10 of 143 CpG sites that remained significant after false discovery rate correction. These CpG sites were localized to eight genes linked to neuronal and developmental pathways, consistent with the neural crest origin of melanoma, as well as epithelial and immune-associated signatures.

Outlook:

Further analyses will focus on highly variable CpG sites in the TCGA SKCM cohort and on relative methylation differences as additional filtering criteria. Validation of these findings in independent published cohorts is planned (**Figure 1**).



ID	Source	Term ID	Term Name	P_{adj} (query_1)
1	GO:BP	GO:0048731	system development	6.886×10^{-6}
2	GO:BP	GO:0001935	endothelial cell proliferation	8.544×10^{-5}
3	GO:BP	GO:0007155	cell adhesion	4.961×10^{-3}
4	GO:BP	GO:0060429	epithelium development	1.704×10^{-2}
5	GO:BP	GO:0048754	branching morphogenesis of an epithelial tube	4.020×10^{-2}
6	GO:CC	GO:0005576	extracellular region	2.328×10^{-2}

Figure 4: Basic differential methylation analysis between responders and non-responders highlights neuronal and developmental pathways consistent with the neural crest origin of melanoma, alongside epithelial and immune-associated signatures.