Label-free and automated approach to rapidly classify microsatellite instability (MSI) in early colon cancer (CC) analyzing the AIO ColoPredictPlus 2.0 (CPP) registry trial.

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Background: MSI due to mismatch repair defects accounts for 15-20% of all CC, has high prognostic and predictive value and is broadly utilized in treatment decisions. Artificial intelligence (AI) integrated, label-free quantum cascade laser (QCL) based infrared (IR) imaging resolves spatial and molecular alterations such as MSI in unstained cancer tissue sections. We aimed to evaluate the method for microsatellite instability/stability (MSI/MSS) classification in samples from the prospective multicenter AIO CPP registry trial. **Methods:** Paraffin-embedded unstained cancer tissue slides from patients (pts.) participating in CPP were measured (avg. 30 min/slide) and analyzed. The cohort was split into training (train), test (test), and validation (vali) sets. Cancer regions were first preselected based on a self-developed convolutional neural network (CNN) CompSegNet (Schuhmacher, medrxiv 2021). A VGG-16 CNN then classified MSI/MSS in these regions. Endpoints were area under receiver operating characteristic (AUROC) and area under precision recall curve (AUPRC). **Results:** 547 pts. (train n=331, test n=69, vali n=147) were analyzed. The baseline characteristics for the sub-cohorts are illustrated in the table. Mutation (MT) status: RAS MT: train 30% / test 30% / vali 37%; BRAF MT: train 27% / test 23% / vali 14%. The preselection of cancer regions reached a validation AUROC of 1.0. The subsequent MSI/MSS classifier reached a validation AUROC of 0.9 and AUPRC of 0.74 (sensitivity 85%, specificity 84%). **Conclusions:** Our multicenter approach using AI integrated label-free IR imaging provides an automated, fast, and reliable classification for MSI/MSS with an AUROC of 0.9 (sensitivity 85%, specificity 84%) almost comparable to the present gold standard immunohistochemistry. The method described here requires less samples for training when compared to other AI approaches which could facilitate the development of prognostic/predictive classifiers in the setting of randomized controlled trials. This novel technique may support further understanding of the increasingly important MSI CC cohort and support treatment decisions e.g. in specific subgroups such as targetable fusions. We expect our approach to be a broadly applicable diagnostic tool in the future. Research Sponsor: Ministry of Culture and Science (MKW) of the State of North-Rhine Westphalia, Germany, Pharmaceutical/Biotech Company.

Baseline characteristics for cohorts.

		train (MSI)	train (MSS)	test (MSI)	test (MSS)	vali (MSI)	vali (MSS)
N		142	189	30	39	26	121
Age	mean	71	68	73	70	73	66
Sex	f/m in %	64/36	40/60	67/33	31/69	65/35	50/50
UICC	I (%)	9 (6)	1 (0)	2 (6)	0 (0)	1 (4)	0 (0)
	II (%)	64 (45)	37 (20)	14 (47)	8 (20)	16 (61)	13 (11)
	III (%)	69 (49)	151 (80)	14 (47)	31 (80)	9 (35)	108 (89)
Location	left (%)	30 (21)	98 (52)	6 (20)	21 (54)	3 (12)	53 (44)
	right (%)	112 (79)	90 (48)	24 (80)	18 (46)	23 (88)	64 (53)
	other (%)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	4 (3)

f: female; m: male.