2329P-Evidence for the utility of artificial intelligence (AI) and image analysis in Ki-67 quantification in solid tumors

Xavier Pichon[1], Rania Gaspo[1], Sabine Iglesias[1], Darshan Kumar[2], Maroua Tliba[1], Renaud Burrer[1] and Amanda Finan[1]

[1]Cerba Research, Montpellier, France and Montreal, Canada; [2]Aiforia Technologies Plc., Helsinki, Finland.

Background

Although it is an important biomarker in oncology (mostly in breast and prostate), Ki-67 immunohistochemistry (IHC) analysis has yet to be standardized. Working groups have provided guidelines for Ki-67 scoring in different cancer types to limit pathologist's variability.[1,2] Digital analysis solutions to assist scoring with image analysis or AI have recently emerged in the evaluation of Ki-67 as rapid and robust solutions. In this context, we compared the results of Ki-67 scoring performed with Aiforia Platform® (AI platform) and Halo® (image analysis supervised software) against three independent pathologists (patho) on various solid tumors.

Method

We stained 192 tumors (Table 1) of various origins including breast and prostate with the CONFIRM anti-Ki-67 clone (30-9) (ROCHE monoclonal primary antibody (IVD)) on the Ventana Benchmark Ultra. Three pathologists were appropriately trained following the International Ki-67 Working Group (IKWG) recommendations and scored tissues accordingly.[3]

Based on deep learning, Aiforia Platform® was able to automatically score Ki-67 positive tumor cells (Ki-67+) within minutes. The random forest classifier from Halo® software was used to separate the image into tumor, non-tumor and background, which was also confirmed by a pathologist. After cell segmentation, Ki-67+ was assessed by thresholding.

A matched pairs statistical analysis was performed with JMP® software.

Organ/Tissue	Histology	Sample size
Stomach	Adenocarcinoma	8
Esophagus	Adenocarcinoma	8
Colon	Adenocarcinoma	8
Liver	Hepatocellular carcinoma	8
Pancreas	Adenocarcinoma	8
Lung	Squamous cell carcinoma / Papillary adenocarcinoma / Small cell carcinoma	8
Cerebrum	Astrocytoma / Glioblastoma	8
Spleen	DLBCL / DBCL	8
Thyroid gland	Papillary adenocarcinoma / Follicular carcinoma	8
Lymph node	Hodgkin's lymphoma / T-cell lymphoma / Anaplastic large cell lymphoma	8
Skin	Squamous cell carcinoma / Dermatofibrosarcoma / Liposarcoma / Malignant melanoma	32
Breast	Invasive ductal carcinoma / Invasive lobular carcinoma	16
Ovary	High grade serous carcinoma / Disgerminoma / Differentiated sertoli cell tumor	16
Uterus	Endometroid adenocarcinoma	8
Cervix	Squamous cell carcinoma	8
Prostate	Adenocarcinoma	8
Testis	Seminoma	8
Kidney	Clear cell carcinoma	8
Bladder	High grade urothelial carcinoma	8

Table 1: Sample size by solid tumor type from the multiple organ tumor tissue microarray (TMA) (n=192).

Workflow

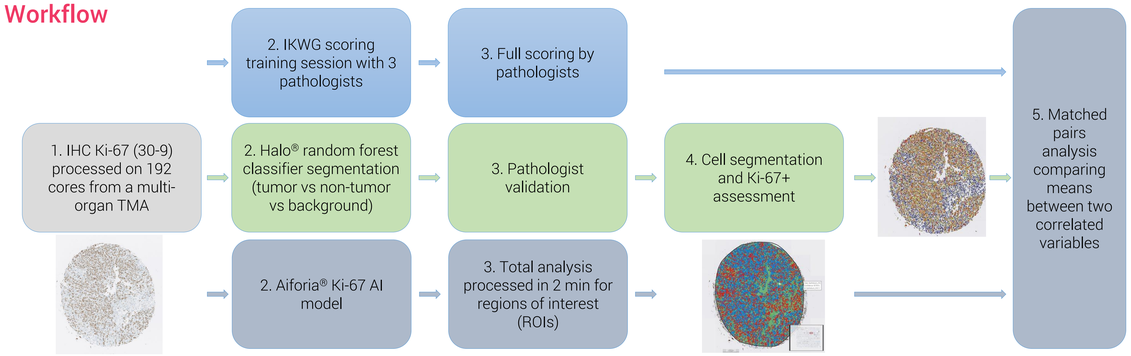

Fig 1. Example of an IHC Ki-67 staining workflow from a lung cancer specimen (papillary adenocarcinoma).

Results: Ki-67 quantification on solid tumors

Out of 192 cores, only 158 were analyzed due to absence of tissue and/or pathologists unable to score. Ki-67+ cells were detected in 24.38 - 28.71% of the tumor cells on average depending on the analysis approach applied (Table 2). Our study shows a very high consistency of results obtained for Ki-67 scoring between the two image analysis softwares, Aiforia® and Halo® (r^2=0.95), on solid tumors analyzed (n=158). The correlation obtained between the pathologists was however weaker (mean r^2=0.83), despite appropriate training and following of guidelines, but remains within an acceptable range (Table 3).

n=158	Mean %Ki-67+	Mean %Ki-67+ with patho	SD
Aiforia®	26.30	/	/
Halo®	24.38	/	/
Pathologist A	27.13	26.49	1.91
Pathologist B	23.63		
Pathologist C	28.71		

Table 2: Ki-67 quantification results on all solid tumors analyzed (n=158).

Results: Matched pairs analysis of Ki-67 quantification on solid tumors

Matched pairs analysis (n=158)	Mean difference of %Ki-67+	p	SEM	p	r^2
Halo-Aiforia	-1.91	0.0024	0.62	0.9988	0.95
A-Aiforia	0.83	0.4852	1.19	0.2426	0.83
B-Aiforia	-2.66	0.0193	1.13	0.9904	0.82
C-Aiforia	2.42	0.0007	0.70	0.0004	0.94
A-Halo	2.75	0.0504	1.39	0.0252	0.76
B-Halo	-0.75	0.4971	1.10	0.7515	0.80
C-Halo	4.33	<0.001	0.89	<0.001	0.89
B-A	-3.5	<0.001	1.34	0.9949	0.78
C-A	1.58	0.1587	1.12	0.0794	0.86
C-B	5.08	<0.001	1.06	<0.001	0.85

Table 3: Matched pairs analysis of Ki-67 quantification results in various solid tumors (n=158). Cell color coding for r^2: green >0.90; orange: 0.90 - 0.80; yellow: 0.80 - 0.75

As indicated in table 3 and figure 2, our study shows the highest consistency of results obtained for Ki-67 scoring between the two image analysis softwares, Aiforia® and Halo® (r^2=0.95). The correlation garnered when matching AI with pathologists was fair to strong (r^2=0.83/0.82/0.94), whereas the correlation when comparing Halo® against pathologists scoring was mostly fair (r^2=0.76/0.80/0.89). However, the correlation obtained between the 3 pathologists was generally weaker (r^2=0.78 for B-A, r^2=0.86 for C-A and r^2=0.85 for C-B), and the weakest link was patho A-Halo (r^2=0.76).

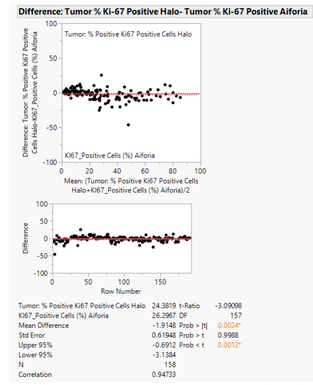

Fig 2: Matched pairs analysis of AI vs Halo® (n=158).

Results: Ki-67 quantification by solid tumor type

	Breast (n=16)			Lung (n=8)			Prostate (n=6)			Cervix (n=8)			Cerebrum (n=6)			Colon (n=6)			Esophagus (n=8)			Kidney (n=3)			Liver (n=5)			Lymph node (n=8)		
	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD
Aiforia	16.53			18.25			3.44			29.36			16.33			38.36			29.96			2.43			11.00			28.95		
Halo	12.84			19.38			5.71			33.86			23.75			31.34			22.77			6.03			14.47			32.87		
Pathologist A	19.64			20.12			4.59			35.38			15.00			36.67			44.38			1.73			12.04			23.13		
Pathologist B	11.63	16.71	3.39	15.83	18.88	2.03	6.33	5.08	0.84	27.75	33.28	3.69	13.33	16.27	2.81	32.00	33.61	2.04	26.88	36.16	6.19	2.33	2.19	0.30	9.40	13.03	3.08	65.00	41.59	15.61
Pathologist C	18.88			20.68			4.37			36.71			20.48			32.15			37.23			2.50			17.64			36.64		
	Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis		
	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2
Halo-Aiforia	-3.69	0.0258	0.98	1.13	0.5461	0.98	2.27	0.3078	0.78	-4.50	0.1188	0.98	7.43	0.0075	0.97	-7.02	0.0215	0.99	-7.19	0.0213	0.92	3.59	0.0744	0.84	3.46	0.0351	0.98	3.92	0.0461	0.98
A-Aiforia	3.11	0.2296	0.88	1.77	0.1887	0.99	1.10	0.6859	0.64	1.51	0.8879	0.32	-1.33	0.5225	0.95	-1.70	0.6702	0.96	14.42	0.0107	0.81	-0.70	0.148	1.00	1.04	0.6611	0.91	-5.82	0.1309	0.93
B-Aiforia	-4.91	0.1006	0.69	-2.42	0.6251	0.91	2.90	0.0344	0.99	-6.11	0.3799	0.63	-2.99	0.2513	0.96	-6.36	0.1325	0.98	-3.08	0.3407	0.87	-0.10	0.882	1.00	-1.60	0.1169	1.00	36.05	0.0042	0.19
C-Aiforia	2.34	0.1228	0.93	2.43	0.3328	0.97	0.93	0.1555	0.97	2.85	0.6842	0.69	4.16	0.0303	0.98	-6.21	0.082	0.99	7.27	0.0743	0.96	0.07	0.8175	1.00	6.64	0.1612	0.97	7.69	0.0245	0.96
A-Halo	6.79	0.0367	0.88	0.63	0.746	0.98	-1.18	0.7735	0.17	6.01	0.5398	0.34	-8.75	0.0119	0.94	5.33	0.2035	0.96	21.60	0.0021	0.78	-4.29	0.0362	0.86	-2.43	0.2086	0.95	-9.75	0.0089	0.95
B-Halo	-1.21	0.5887	0.67	-0.36	0.4109	0.90	0.62	0.7622	0.73	-1.61	0.7772	0.61	-10.42	0.0048	1.00	0.66	0.8186	0.97	4.10	0.1383	0.90	-3.69	0.0426	0.86	-5.07	0.0211	0.98	32.13	0.0068	0.31
C-Halo	6.04	0.0039	0.88	1.30	0.4803	0.98	-1.35	0.4895	0.79	7.35	0.3043	0.66	-3.27	0.0243	0.99	0.81	0.6846	0.99	14.45	0.0122	0.95	-3.53	0.0736	0.89	-3.17	0.4692	0.95	3.77	0.1234	0.87
B-A	-8.01	0.0589	0.60	-4.18	0.3435	0.95	1.80	0.4943	0.64	-7.63	0.1492	0.89	-1.67	0.5558	0.94	-4.67	0.1479	0.99	-17.50	0.0007	0.91	0.60	0.1835	1.00	-2.64	0.2853	0.94	41.88	0.0013	0.47
C-A	-0.76	0.8084	0.80	0.67	0.6966	0.98	-0.17	0.9453	0.71	1.34	0.8501	0.72	5.48	0.047	0.95	-4.52	0.3551	0.95	-7.15	0.2657	0.74	0.77	0.2518	1.00	5.60	0.347	0.84	13.51	0.0005	0.96
C-B	7.25	0.0013	0.87	4.85	0.2261	0.96	-1.97	0.0578	0.96	8.96	0.0479	0.93	7.15	0.0452	0.98	0.15	0.9586	0.96	10.35	0.0553	0.89	0.17	0.8497	1.00	8.24	0.1453	0.97	-28.36	0.0101	0.44
		mean r^2	0.81		mean r^2	0.96		mean r^2	0.74		mean r^2	0.68		mean r^2	0.97		mean r^2	0.97		mean r^2	0.87		mean r^2	0.94		mean r^2	0.95		mean r^2	0.72
		SD	0.10		SD	0.03		SD	0.16		SD	0.17		SD	0.02		SD	0.01		SD	0.06		SD	0.06		SD	0.04		SD	0.29

	Spleen (n=7)			Stomach (n=8)			Testtis (n=5)			Ovary (n=12)			Skin (n=25)			Pancreas (n=7)			Bladder (n=8)			Thyroid (n=8)			Uterus (n=8)		
	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD	Mean % Ki67 positivity cells	Mean 3 patho	SD
Aiforia	65.92			46.68			29.41			27.90			32.31			21.54			13.51			1.79			32.13		
Halo	68.25			36.43			21.29			24.61			29.26			23.01			12.83			2.89			28.96		
Pathologist A	64.29			53.75			44.20			34.17			18.80			27.86			11.50			3.29			44.38		
Pathologist B	57.71	64.62	4.83	44.38	49.11	3.16	25.80	36.33	7.02	25.00	30.23	3.48	20.40	23.85	5.66	19.29	21.70	4.10	8.33	11.53	2.16	2.13	2.82	0.46	29.00	35.75	5.75
Pathologist C	71.87			49.21			39.00			31.51			32.35			17.97			14.77			3.04			33.86		
	Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis			Matched pairs analysis		
	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2	Mean difference	p	r^2
Halo-Aiforia	2.34	0.1887	0.96	-10.25	0.1144	0.74	-8.13	0.0154	0.99	-3.29	0.2082	0.95	-3.05	0.0169	0.98	1.48	0.7833	0.83	-0.68	0.289	0.99	1.10	0.089	0.61	-3.17	0.0673	0.99
A-Aiforia	-1.63	0.7317	0.56	7.07	0.3063	0.81	14.79	0.2033	0.77	6.27	0.0642	0.94	-13.50	0.001	0.73	6.32	0.1342	0.93	-2.01	0.1194	0.99	1.50	0.1145	0.92	12.24	0.0013	0.97
B-Aiforia	-8.20	0.0265	0.83	-2.31	0.3718	0.97	-3.61	0.2819	0.96	-2.90	0.1261	0.98	-11.91	0.0001	0.86	-2.25	0.4151	0.96	-5.17	0.1105	0.95	0.34	0.2881	0.80	-3.13	0.359	0.95
C-Aiforia	5.95	0.0885	0.85	2.53	0.405	0.95	9.59	0.1014	0.94	3.61	0.0016	0.99	0.04	0.9859	0.89	-3.57	0.3919	0.91	1.26	0.3146	0.98	1.25	0.0038	0.89	1.73	0.5801	0.95
A-Halo	-3.97	0.4734	0.49	17.32	0.078	0.63	22.91	0.0932	0.74	9.55	0.0751	0.85	-10.45	0.0113	0.66	4.84	0.5594	0.64	-1.33	0.3355	0.97	0.40	0.6442	0.79	15.42	0.0018	0.96
B-Halo	-10.54	0.0065	0.93	7.94	0.1954	0.82	4.51	0.1694	0.94	0.39	0.7469	0.99	-8.86	0.0024	0.79	-3.73	0.6009	0.68	-4.49	0.1183	0.92	-0.76	0.1817	0.69	0.04	0.9893	0.93
C-Halo	3.62	0.1776	0.91	12.78	0.1178	0.65	17.71	0.0372	0.91	6.90	0.0191	0.96	3.09	0.2846	0.82	-5.04	0.1861	0.91	1.94	0.2819	0.96	0.15	0.8443	0.39	4.90	0.1484	0.93
B-A	-6.57	0.1842	0.47	-9.38	0.1303	0.86	-18.40	0.1127	0.85	-9.17	0.0528	0.89	1.60	0.5357	0.86	-8.57	0.0057	0.99	-3.17	0.1634	0.96	-1.16	0.2879	0.73	-15.38	0.0051	0.95
C-A	7.59	0.1634	0.58	-4.54	0.3829	0.89	-5.20	0.3941	0.94	-2.66	0.4352	0.93	13.54	0.0002	0.79	-9.89	0.2072	0.72	3.27	0.0451	0.99	-0.25	0.773	0.82	-10.51	0.0444	0.91
C-B	14.16	0.0016	0.96	4.84	0.1604	0.95	13.20	0.0526	0.96	6.51	0.0054	0.98	11.95	0.0001	0.86	-1.31	0.8307	0.75	6.43	0.0903	0.91	0.91	0.0987	0.65	4.86	0.0367	0.97
		mean r^2	0.75		mean r^2	0.83		mean r^2	0.90		mean r^2	0.95		mean r^2	0.82		mean r^2	0.83		mean r^2	0.96		mean r^2	0.73		mean r^2	0.95
		SD	0.18		SD	0.10		SD	0.07		SD	0.03		SD	0.07		SD	0.11		SD	0.02		SD	0.12		SD	0.02

Table 4: Results of Ki-67 quantification by solid tumor type with associated matched pairs analysis. Cell color coding for r^2: green >0.90; orange: 0.90 - 0.80; yellow: 0.80 - 0.75

Overall, for lung, cerebrum, colon, bladder and uterus, correlations between Aiforia Platform-Halo and inter-pathologist comparisons were consistent and high (r^2>0.90). Depending on the organ, there were significant variations between pathologists, whereas the scores obtained with the image analysis softwares remain close. For instance, with lymph nodes Ki-67 scoring, a 0.98 correlation was found between Aiforia Platform and Halo while the inter-pathologists' correlation was only 0.44 (C-B). Of particular mention, out of the 19 primary tumors analyzed, only stomach cancer showed correlations of less than 0.75 between the image analysis softwares (r^2=0.74) with inter-pathologists' correlation still high (ranging from 0.86-0.95).

Conclusion

This work shows that recent AI-based image analysis tools such as Aiforia® and image analysis supervised software like Halo® provide valuable assistance in the field of image analysis and allow to drastically reduce inter-pathologist variability in the Ki-67 scoring of solid tumors.

References:

[1] Polley MY et al. An international study to increase concordance in Ki-67 scoring. Mod Pathol. 2015 Jun;28(6):778-86. doi: 10.1038/modpathol.2015.38.

[2] Nielsen TO et al. Assessment of Ki-67 in Breast Cancer: Updated Recommendations From the International Ki-67 in Breast Cancer Working Group. J Natl Cancer Inst. 2021 Jul 1;113(7):808-819. doi: 10.1093/jnci/djaa201.

[3] Welcome to Ki-67-QC calibrator. URL [http://www.gpec.ubc.ca:8080/tmadb-0.1/calibrator/index]

Disclosures: XP, SI, MT, RB & AF are Cerba Research employees. RG is a consultant and DK is an Aiforia Technologies Plc employee. Study is sponsored by Cerba Research.

xpichon@cerbaresearch.com