Sentinel-10™- A new multi-cancer early detection test

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INTRODUCTION

Multi-cancer early detection (MCED) test that can identify cancer from a liquid biopsy, and can be used across various cancer types is an unmet clinical need. Tumors shed DNA into body fluids and therefore DNA methylation analysis of cell-free DNA from blood can be used for minimally invasive cancer tests. Sentinel-10™ liquid biopsy is based on a novel set of 10 biomarker loci (Fig 1, Ref 1, 2) hypermethylated in 10 common carcinoma types (marked purple in Table 1). We previously demonstrated that Sentinel-10™ can detect lung and pancreatic cancers (Refs 2, 3). Here, we present a new bioinformatics analysis revealing the performance of Sentinel-10™ in additional cancer types and in a clinical blood cohort of patients with breast cancer.

MATERIALS AND METHODS

DNA methylation data from Illumina microarray platforms from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were downloaded from respective databases, normalized and analyzed as previously described (Ref 1) in R environmet ver 4.2.0. Sentinel-10™ DNA methylation of cancer samples was tested against three control cohorts. Control Blood cohort consists of 1388 samples from two GEO datasets (GSE40279, GSE87571). NT.TCGA cohort consist of all 746 non-tumor tissue samples from TCGA database. NT.GEO cohort consist of 796 normal tissue samples from 16 GEO datasets (GSE50192, GSE48472, GSE48684, GSE61278, GSE61258, GSE63704, GSE79100, GSE64509, GSE63315, GSE51954, GSE51954, GSE61259, GSE60655, GSE64490, GSE61257, GSE70977).
Sentinel-10™ liquid biopsy test was performed by DNA methylation specific qPCR described before (Ref 2) that analyzed cfDNA extracted from plasma samples obtained from healthy controls and breast cancer cases. Cancer cohort consisted of 15 women diagnosed with breast cancer, 14 stage IV cases and 1 stage II case; median age 66 (range 42-85); 13 white and 2 hispanic/latino women.

Fig 1

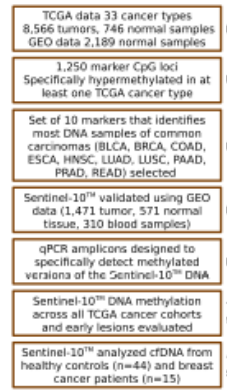

Fig 1 The workflow of the Sentinel-10™ evolution

Table 1

Acronym	TCGA Cancer Type Name	AUC Blood	AUC NT.TCGA	AUC NT.GEO
ACC	Adrenocortical carcinoma	0.942	0.695	0.837
BLCA	Bladder Urothelial Carcinoma	0.999	0.982	0.995
BRCA	Breast invasive carcinoma	0.999	0.984	0.996
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	1.000	0.995	1.000
CHOL	Cholangiocarcinoma	0.998	0.977	0.992
COAD	Colon adenocarcinoma	1.000	0.993	0.999
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	0.995	0.965	0.984
ESCA	Esophageal carcinoma	1.000	0.991	0.999
GBM	Glioblastoma multiforme	0.996	0.957	0.985
HNSC	Head and Neck squamous cell carcinoma	1.000	0.992	0.999
KICH	Kidney Chromophobe	0.881	0.544	0.732
KIRC	Kidney renal clear cell carcinoma	0.984	0.893	0.952
KIRP	Kidney renal papillary cell carcinoma	0.940	0.892	0.941
LAML	Acute Myeloid Leukemia	0.964	0.807	0.904
LGG	Brain Lower Grade Glioma	0.991	0.932	0.972
LIHC	Liver hepatocellular carcinoma	0.989	0.935	0.971
LUAD	Lung adenocarcinoma	0.999	0.984	0.996
LUSC	Lung squamous cell carcinoma	0.998	0.977	0.993
MESO	Mesothelioma	0.987	0.907	0.959
OV	Ovarian serous cystadenocarcinoma	0.871	0.567	0.740
PAAD	Pancreatic adenocarcinoma	0.994	0.954	0.981
PCPG	Pheochromocytoma and Paraganglioma	0.746	0.785	0.501
PRAD	Prostate adenocarcinoma	0.998	0.978	0.992
READ	Rectum adenocarcinoma	1.000	0.993	1.000
SARC	Sarcoma	0.938	0.716	0.847
SKCM	Skin Cutaneous Melanoma	0.968	0.796	0.893
STAD	Stomach adenocarcinoma	1.000	0.993	0.999
TGCT	Testicular Germ Cell Tumors	0.798	0.577	0.631
THCA	Thyroid carcinoma	0.909	0.546	0.756
THYM	Thymoma	0.829	0.625	0.620
UCEC	Uterine Corpus Endometrial Carcinoma	0.998	0.988	0.996
UCS	Uterine Carcinosarcoma	0.999	0.979	0.995
UVM	Uveal Melanoma	0.736	0.827	0.530

Table 1 - TCGA cancer cohorts:
The last tree columns show areas under the curve (AUC) for each cancer type, when DNA methylation from Sentinel-10™ loci is used to test respective cancer cohort and three independent control cohorts. The original 10 carcinoma types are marked purple and the additional 10 carcinoma types that have all three AUCs > 0.9 are marked blue.

RESULTS

The bioinformatics analysis of all 33 TCGA cancer cohorts revealed that the Sentinel-10™ biomarker loci are predominantly hypermethylated in 10 additional cancer types (marked blue in Table 1). Therefore, the Sentinel-10™ MCED test has the potential to detect 20 cancer types according to TCGA classification with high sensitivity and specificity (Fig 2, Table 1). These 20 TCGA cancer types account for 73.7% of new cancer cases and 80.5% of cancer deaths worldwide (Table 2).

Sentinel-10™ loci are also hypermethylated in independent hepatocellular carcinoma, mesothelioma, and cholangiocarcinoma cohorts from GEO (Fig 3). Furthermore, the Sentinel-10™ loci are hypermethylated early in cancer progression in bile duct and prostate early lesions (Fig 3) and in breast, colorectal, oesophageal, lung and pancreatic early lesions (Ref 4). Therefore Sentinel-10™ has potential to detect early cancer stages as soon as tumor DNA becomes present in blood or other body fluids.

The pilot clinical study (Figs 4 and 5), shows that the Sentinel-10™ liquid biopsy test can differentiate between blood from metastatic breast cancer cases and blood from cancer free controls with high sensitivity and specificity (AUC=0.982, 95% CI: 0.952-1.0).

CONCLUSIONS

- **Sentinel-10™ has the potential to detect the majority of cancers.**
- **Sentinel-10™ can possibly detect early stages of cancers.**
- **The Sentinel-10™ liquid biopsy test detects breast cancer in blood samples.**
- **Sentinel-10™ represents an innovative MCED test for cancer patients.**

Fig 2

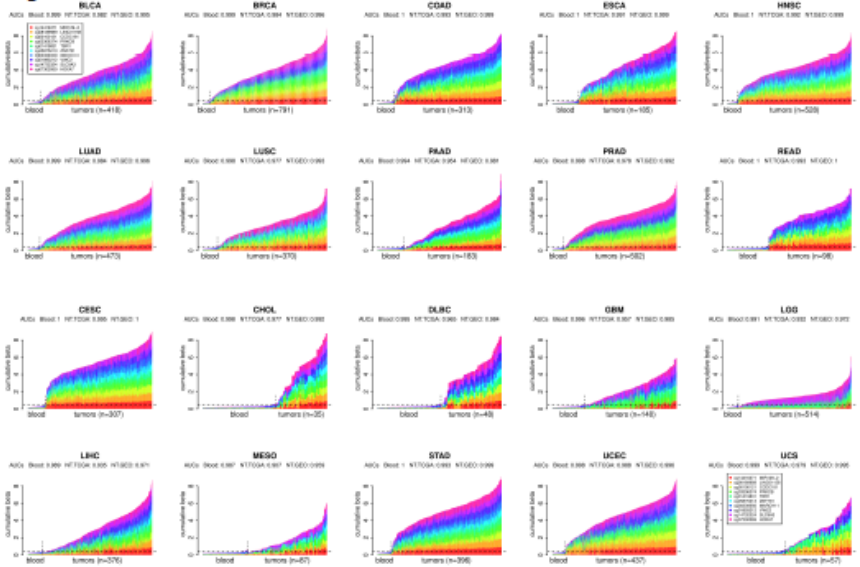

Fig 2 Sentinel-10™ DNA methylation in 20 TCGA cancer cohorts. First two rows represent cancers for which Sentinel-10™ was originally designed and the last two rows represent cancers where Sentinel-10™ would be also applicable based on the bioinfomatics analysis. The plots represent DNA methylation of individual Sentinel-10™ loci in individual cancer samples. A representative sample of 50 control blood samples is shown for comparison. Horizontal dashed lines represent 95th percentile of cumulative DNA methylation of the entire control blood cohort (n=1388).

Fig 3

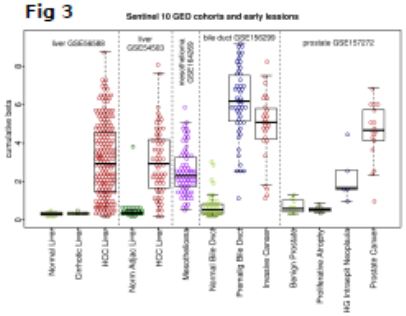

Fig 3 Sentinel-10™ DNA methylation in independent data from GEO cohorts. Boxplots present cumulative DNA methylation of Sentinel-10™ loci in cohorts of cancer samples, normal samples and early precancerous lesions from 5 GEO datasets.

Fig 4

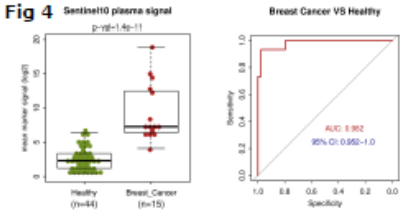

Fig 4 The mean Sentinel-10™ signal in plasma from the pilot study healthy control cohort and cohort of breast cancer cases, boxplots left, p-value from Wilcoxon rank sum test, ROC analysis right.

Table 2 - World-wide numbers of new cancer cases and deaths according to Global Cancer Statistics 2020 (Ref 5). Cancers potentially detectable by Sentinel-10™ are marked green, the total proportion of cancer cases and cancer death detectable by Sentinel-10™ is listed at the bottom.

Table 2

Cancer Site	New Cases	New Deaths
Anus	50,865	19,293
Bladder	573,278	212,536
Brain, nervous system	308,102	251,329
Cervix uteri	604,127	341,831
Colon	1,148,515	576,858
Corpus uteri	417,367	97,370
Esophagus	604,100	544,076
Female breast	2,261,419	684,996
Gallbladder	115,949	84,695
Hodgkin lymphoma	83,087	23,376
Hypopharynx	84,254	38,599
Kaposi sarcoma	34,270	15,086
Kidney	431,288	179,368
Larynx	184,615	99,840
Leukemia	474,519	311,594
Lip, oral cavity	377,713	177,757
Liver	905,677	830,180
Lung	2,206,771	1,796,144
Melanoma of skin	324,635	57,043
Mesothelioma	30,870	26,278
Multiple myeloma	176,404	117,077
Nasopharynx	133,354	80,008
Non-Hodgkin lymphoma	544,352	259,793
Nonmelanoma of skin	1,196,073	63,731
Oropharynx	98,412	48,143
Ovary	313,959	207,252
Pancreas	495,773	466,003
Penis	36,068	13,211
Prostate	1,414,259	375,304
Rectum	732,210	339,022
Salivary glands	53,583	22,778
Stomach	1,089,103	768,793
Testis	74,458	9,334
Thyroid	586,202	43,646
Vagina	17,908	7,995
Vulva	45,240	17,427
All sites	19,292,789	9,958,133
Sentinel10 sites	14,214,271	8,014,860
Sentinel10 percent of all	73.7 %	80.5 %

Fig 5

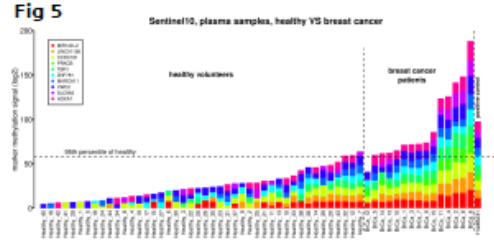

Fig 5 The DNA methylation signals from individual Sentinel-10™ markers obtained from the pilot clinical study. Y-axis is in log2 scale.

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