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Brief communication

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Tumor fraction-based prognostic tool for cancer patients referred to early phase clinical trials

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Selecting patients for phase I cancer trials is crucial to ensure a sufficient life expectancy. Frail patients, better suited for palliative care, should not be exposed to new drugs with minimal benefit. Enrolling patients at high risk of early death can jeopardize the study. Our analysis of two large precision medicine studies used tumor fraction from ctDNA to develop a predictive model, demonstrating notable predictive accuracy and aiding in patient selection.

Phase I trials primarily delineate the toxicity profile and establish the appropriate dosage levels of novel drugs or combinations in preparation for Phase II/III studies. A significant challenge for Phase I investigators is determining which patients should be offered entry into these trials. Typically, patients chosen for Phase I oncology studies possess a life expectancy exceeding three months. However, a concerning 15-20% succumb within 90 days of inclusion[1–3]. At present, there is a glaring absence of objective and consistently reproducible biomarkers for improving patient selection. While the Royal Marsden Hospital (RMH)[1] and the Gustave Roussy Immune (GRIM)[4] scores, both validated and equipped with three variable metrics (RMH: albumin, lactate, and number of metastatic sites, GRIM: albumin, lactate, and neutrophil/lymphocyte ratio), offer some guidance, the precision is far from ideal.

The use of liquid biopsies is progressively becoming a standard in the clinical guidelines for treating advanced-stage cancer patients[5–7]. Different designs exist for these ctDNA analyses, with some concentrating on specific gene alterations while others conduct a more extensive analysis of the cancer genome[6,8]. An interesting technological potential of extensively analyzing ctDNA lies in determining the tumor fraction (TF) by assessing the amount of ctDNA released. In a study by Stover and colleagues, TF was calculated in metastatic breast cancer patients by evaluating genomic aneuploidy[9]. The study discovered that a TF of 10% or more independently indicated prognosis when adjusted for clinical and pathological factors, presenting a hazard ratio (HR) of 2.14 with a 95% confidence interval (CI) between 1.4 and 3.8 ($P < 0.001$). Other studies in different tumor types highlighted that a higher TF usually indicates a more severe prognosis[10–12].

In this context, our investigation aims to determine if using a TF biomarker to quantify ctDNA, derived from commonly used commercial liquid biopsy tests, can provide substantial prognostic data to refine patient selection in early-phase studies.

Patient cohort characteristics

The development cohort encompassed 965 patients diagnosed with advanced solid tumors and enrolled in the BIP study between December 2020 and December 2021 (see supplementary methods for details). Similarly, the validation cohort included 947 patients with analogous diagnoses and enrolled in the STING study during the identical timeframe. For an in-depth look at the clinicopathological and survival attributes of both cohorts, refer to Table 1 and Supplementary Table 1. The median duration of follow-up for the BIP cohort was 17.1 months (IQR 16.3–17.7), whereas for the STING cohort, it was 9.9 months (IQR 9.4–10.5). In terms of median overall survival (OS), the BIP cohort registered 11.5 [10.5–12.7] months, and the STING cohort noted 11.8 [10.3–14.8] months. When evaluated at the 3-month mark, the OS rate for BIP stood at 87.6% (95% CI 85.5–89.7) and 86.1% (95% CI 83.9–88.3) for STING. A noteworthy correlation between TF and OS was observed across both cohorts (as visualized in Fig. 1).

Model formulation and evaluation

Within the BIP cohort, each potential variable (namely TF, albumin, LDH, metastatic site count, and NLR) exhibited a marked association with OS, as detailed in Supplementary Table 2. Delving into the refined multivariate model, several factors were pinpointed: an elevated TF, reduced albumin,

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Table 1 | Characteristics of patients in the development (BIP) cohort and the validation (STING) cohort

	Development cohort (BIP) *n* = 965	Validation cohort (STING) *n* = 947	*p* value[a]
Age (years)	66 (21–93)	63 (19–91)	0.001
Sex			0.11
Female	468 (48.5%)	423 (44.7%)	
Male	497 (51.5%)	524 (55.3%)	
Albumin			0.05
<35 g/L	41 (4.2%)	59 (6.2%)	
≥35 g/L	919 (95.2%)	869 (91.8%)	
Missing data	5 (0.5%)	19 (2%)	
LDH			0.07
>ULN	700 (72.5%)	649 (68.5%)	
≤ULN	215 (22.3%)	244 (25.8%)	
Missing data	50 (5.2%)	54 (5.7%)	
NLR			0.03
<6	713 (73.9%)	310 (32.7%)	
≥6	248 (25.7%)	142 (15.0%)	
Missing data	4 (0.4%)	495 (52.3%)	
Metastatic sites			<0.001
>2	243 (25.2%)	367 (38.8%)	
≤2	715 (74.1%)	580 (61.2%)	
Missing data	7 (0.7%)	0 (0%)	
Tumor fraction			0.02
<10%	706 (73.2%)	644 (68%)	
≥10%	259 (26.8%)	303 (32%)	
Location of the primary			<0.001
NSCLC	120 (12.4%)	152 (16%)	
CRC	136 (14.1%)	101 (10.7%)	
Prostate	130 (13.5%)	88 (9.3%)	
Breast	109 (11.3%)	61 (6.4%)	
Pancreas	102 (10.5%)	65 (6.8%)	
Urothelial	51 (5.3%)	48 (5.1%)	
Biliary tract	29 (3%)	44 (4.6%)	
Ovary	46 (4.8%)	23 (2.4%)	
Kidney	45 (4.7%)	15 (1.6%)	
STS	26 (2.7%)	29 (3.1%)	
Gastric/ oesogastric junction	21 (2.2%)	28 (3%)	
Head and neck	7 (0.7%)	38 (4%)	
Melanoma	12 (1.2%)	27 (2.9%)	
Cervix	24 (2.5%)	13 (1.4%)	
Uterus	23 (2.4%)	11 (1.2%)	
SCLC	2 (0.2%)	25 (2.6%)	
Other	80 (8.3%)	160 (16.9%)	
NA	2 (0.2%)	19 (2%)	

Data are mean (SD) or *n* (%).

LDH lactate dehydrogenase, *NLR* neutrophil-to-lymphocyte ratio, *NSCLC* non-small-cell lung cancer, *CRC* colorectal cancer, *STS* soft-tissue sarcoma, *SCLC* small-cell lung cancer, *NA* not available.

[a]Unpaired Student's t-test for unequal variances, Pearson's Chi-squared test or Fisher's exact test.

the existence of 3 or more metastatic sites, and a heightened NLR all correlated with decreased OS. The 3-month AUROC curve registered at 79.87 [95%CI: 75.40–84.34]. Upon inspecting the calibration plots (refer to Supplementary Fig. 1A), it became evident that the model's estimation leaned towards an overestimation for those with lower OS probabilities yet aligned accurately for patients showcasing survival probabilities exceeding 75%. The 3-month Brier score, landing at 0.091, indicates commendable accuracy.

Model validation process

In scrutinizing the STING cohort, the 3-month AUROC was recorded at 76.11 [95%CI: 69.31–82.90]. The calibration plots (highlighted in Supplementary Fig. 1B) revealed certain discrepancies. For patients with lower OS probabilities, the multivariate model tended to underestimate. Conversely, for those with elevated OS probabilities, especially between the 60% and 75% range, an overestimation was evident. Nevertheless, the 3-month Brier score remained at a respectable 0.093.

Nomogram construction for predictive scoring

Using the refined multivariate survival model, we developed a comprehensive nomogram designed to predict three-month survival probabilities (Supplementary Table 3). This predictive tool integrates several key variables, each weighted according to their prognostic significance derived from the final multivariate analysis. The variables included are:

- Tumoral fraction (TF): Assigned a score of 0 for TF < 10% and 64 for TF ≥ 10%, reflecting its strong prognostic impact.
- Albumin levels: Low albumin is scored at 100 due to its high correlation with poor outcomes, while normal levels are scored at 0.
- Metastatic sites: Patients with ≤2 sites are scored at 0, and those with >2 sites are scored at 28, indicating increased risk with more extensive disease.
- Neutrophil-to-lymphocyte ratio (NLR): A score of 0 is given for NLR < 6, and 33 for NLR ≥ 6, highlighting its relevance in inflammation-related prognosis.

For instance, a patient with high TF (≥10%), low albumin, more than two metastatic sites, and a high NLR would accumulate a score of 225 points, correlating with a three-month survival probability of approximately 25%. Conversely, a patient with all favorable parameters would score 0 points, aligning with a survival probability nearing 90% (Supplementary Fig. 3)

Supplementary analyses

Further analyses were conducted to compare the predictive performance of the comprehensive model against simpler models integrating TF with established scores such as the GRIm and RMH scores. The results, detailed in Supplementary Tables 4 and 5, reveal that:

- The AUROC for the model combining TF and the GRIm score was 73.87 in the BIP cohort and 72.69 in the STING cohort, which are both lower than those achieved by our comprehensive model.
- Similarly, the model incorporating TF and the RMH score produced an AUROC of 71.91 for BIP and 66.47 for STING, further substantiating the superiority of the comprehensive model.
- Brier scores were also consistently better in the comprehensive model (0.091 for BIP and 0.093 for STING) compared to the simpler models, indicating better overall prediction accuracy.

Prognostic value of TF is independent of tumor volume

Our findings demonstrate that TF was independently predictive of OS, regardless of the RMS score, which considers the number of metastatic sites. This indicates that TF's predictive value is not contingent on tumor burden. To verify this hypothesis, we examined the relationship between TF and volume by analyzing the volume of all visible lesions with a diameter greater than 1 mm on baseline CT scans (refer to the "Methods" section). The mean tumor volume was found to be 253 cm^3(range 4–2056). The prevalence of

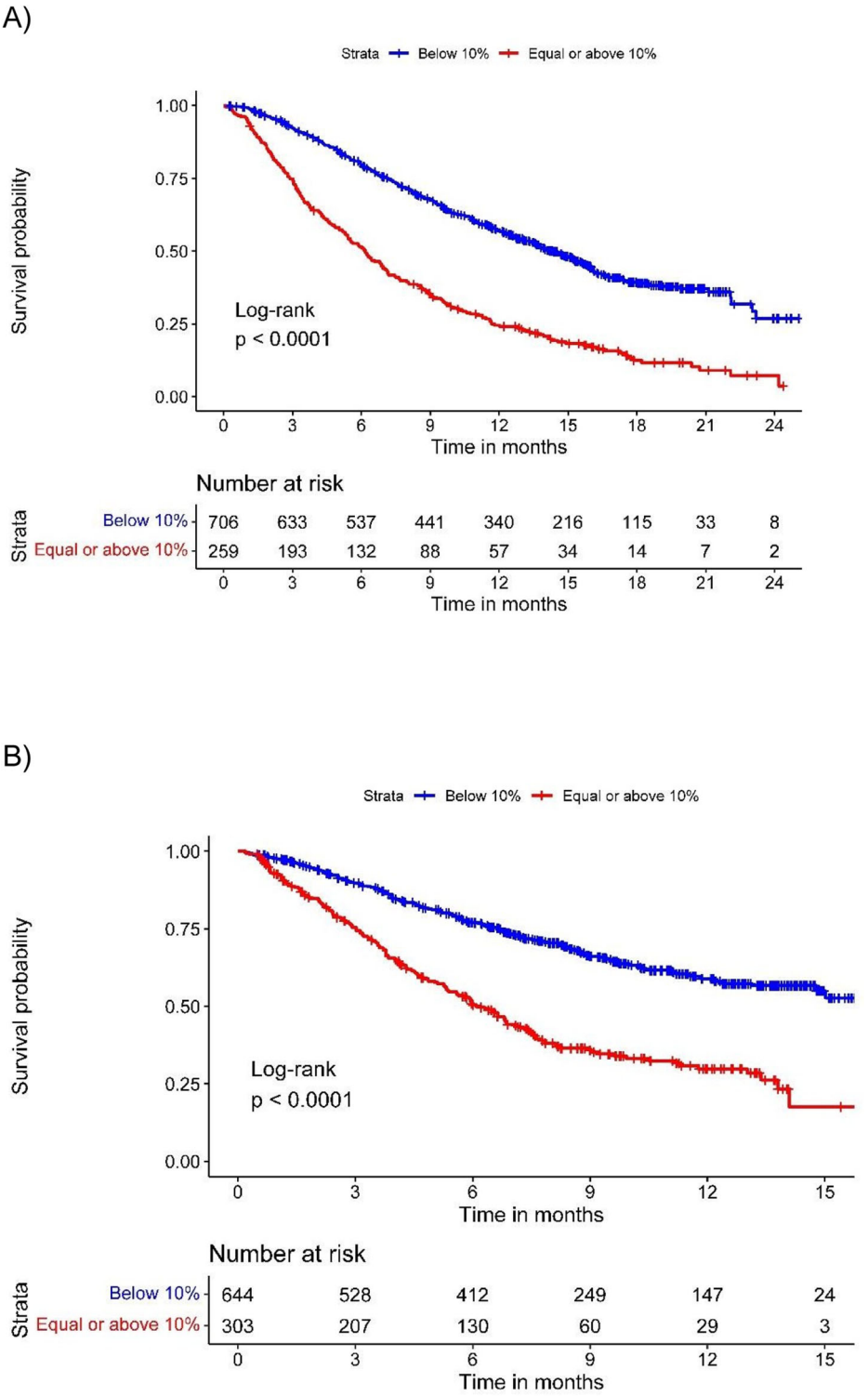

Fig. 1 | Prognostic impact of tumor fraction in patients referred for early phase trials. Kaplan-Meier survival curves for the BIP cohort (**A**) and the STING cohort (**B**), stratified by tumoral fraction.

high TF did not significantly differ between patients with high versus low tumor burden (44.4% vs. 40.5%, $p = 0.65$), suggesting that ctDNA shedding in patients with metastatic disease is more influenced by intrinsic tumor biology than by tumor volume (Supplementary Fig. 4).

Discussion

We propose here a prognostic tool developed thanks to the analysis of two large independent prospective cohorts, with various advanced solid tumors and sample sizes close to 1000 patients. The variables that are incorporated in the model ctDNA levels, albumin, NLR, and metastatic sites are easy to assess in daily clinical practice. We deliberately decided to use all variables as binary to enhance the feasibility of the tool. This however resulted in observed stepwise (rather than perfectly smooth-line) calibration plots, but importantly model calibration as measured using the Brier score remains very favorable.

Modern phase 1 trials demonstrate therapeutic benefits for up to 50% of patients, underscoring the positive impact of recent advancements in cancer treatment[13–15]. In high-volume centers, the demand for enrollment in

Phase I trials frequently outstrips the available capacity. This necessitates a rigorous selection process among a multitude of eligible candidates to allocate the limited study slots. The overarching objective in this process is to discern those patients most likely to adhere to the trial parameters and potentially derive therapeutic benefits. While prevailing selection methodologies are predominantly anchored in individual physician's clinical acumen, we contend that the integration of our nomogram can provide invaluable predictive insights. To foster a more efficient and evidence-based selection process, we have developed the TIMES app (https://acrombe.shinyapps.io/TIMES_test/). This tool facilitates the efficient assimilation of patient data to yield predictions on 3- and 6-month survival probabilities, paving the way for more refined patient selection in future phase I trials.

Methods

Study design and participants

The research utilized data from patients enrolled between December 2020 and December 2021 in two ongoing French precision medicine studies, BIP (NCT02534649, sponsor: Institut Bergonié, Bordeaux, France) and STING (NCT04932525, sponsor: Gustave Roussy, Villejuif, France). Patients considered for these two precision medicine studies were typically referred for eligibility assessment in a phase 1 clinical trial. They were then enrolled to obtain an extensive molecular profile, assisting the phase 1 teams in identifying suitable clinical trials for them. The main inclusion criteria targeted individuals 18 years or older diagnosed with advanced solid tumors or hematological malignancies not suitable for localized treatment. At the point of inclusion, a blood sample was taken for conventional biological metrics, and the number of metastatic sites was determined after central review of CT-scan. The BIP and STING studies comply with regulatory requirements and adhere to Good Clinical Practice guidelines and the Declaration of Helsinki. Both study protocols received approval from independent ethics committees (BIP: Comité de Protection des Personnes Sud-Ouest et Outre Mer III; STING: Comité de Protection des Personnes SUD MEDITERRANEE V). Additionally, each participant provided written informed consent.

Tumor fraction estimation

All participating patients underwent comprehensive genomic profiling with the FDA-approved FoundationOne® Liquid CDx assay, the technical details of which have been previously described[1]. For each sample, ctDNA levels were quantified using a combined TF approach[2], which integrates two distinct methods for TF estimation[3]. When TF is high (typically >10%), it is derived from an assessment of tumor aneuploidy that considers deviations in genome-wide coverage[4]. This method ensures elevated TF is not mistakenly inferred due to high variant allele frequencies of germline variants. Conversely, in the absence of significant tumor aneuploidy (commonly at lower TF levels), TF is computed based on the most prevalent non-germline variant, excluding specific clonal hematopoiesis-linked changes. In the main analyses of this research, TF was categorized binarily, signifying whether a sample had TF ≥ 10% or TF < 10%. This threshold was chosen in line with prior research studies[5,6].

Procedures

Clinical, biological, and TF levels were meticulously compiled from individual patient Case Report Forms. For the nomogram, the candidate variables were sourced from the previously established RMH and GRIM scores. Both participating institutions, Institut Bergonié and Institut Gustave Roussy, granted research approval through their respective Institutional Review Boards.

Total tumor volume

Total tumor volume (TTV) estimation was performed utilizing baseline computed tomography (CT) scans across three planes, meticulously including all discernible lesions exceeding 1 mm in largest diameter. These lesions were precisely outlined on their most substantial axial surface slice, with specific notation of the tumor location. The delineation process was executed manually by two seasoned radiologists employing the SPYD 2D annotation tool, developed by Owkin[16]. Lymph nodes, particularly those with a short axis under 15 mm, were delineated at their largest diameter when deemed pathologically suspect. While bone lesions were included in the comprehensive tumor assessment, pleural and peritoneal effusions were deliberately omitted. The calculation of each lesion's approximate volume was derived from a mathematical model that integrates both the surface area and the minor axis. Consequently, the TTV was meticulously extracted for each patient enrolled in the STING study, offering a critical metric for evaluating tumor burden.

Statistical analysis

The desired endpoint for the nomogram was clearly defined as OS, encompassing death from any cause. We chose statistical tools apt for the data's nature, using the mean and standard deviation or median and inter-quartile range to describe continuous variables. The OS was visually represented using Kaplan-Meier survival curves. The Cox proportional hazards models played a pivotal role in identifying critical prognostic factors. Multivariate analyses incorporated the following factors: age, gender, tumor type, the Gustave Roussy Immune Score (GRIM, including albumin, lactate, and the neutrophil/lymphocyte ratio)[4], the Royal Marsden Hospital (RMH) Score (encompassing albumin, lactate, and the number of metastatic sites)[1], total tumor volume, TF, age, sex, number of previous lines of treatment, and performance status. Hazard ratios (HR), 95% confidence intervals (CI), and p-values for each factor in the multivariate analyses were also calculated, with p-values below 0.05 deemed statistically significant.

The final prediction model's efficiency was gauged both internally (within the same dataset) and externally (using an independent dataset). The AUROC curve's benchmarks offered insights into its interpretation, and calibration was undertaken using predicted probabilities. We also employed the Brier score to assess the global accuracy of predictions (ranging from 0 to 1, with 0 meaning perfect calibration, and the lower the Brier Score, the better the model calibration).

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available due to the clinical and confidential nature of the material but can be made available from the corresponding author on reasonable request.

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Author contributions

Concept and design**:** Antoine Italiano. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: Antoine Italiano. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: Carine Bellera, Kilian Trin. Administrative, technical, or material support: Antoine Italiano. Validation: all authors. Supervision: Antoine Italiano.

Competing interests

A.I.: research grants and advisory board BAYER, BMS, CHUGAI, DAIICHI-SANKYO, IPSEN, MERCK, MSD, NOVARTIS, PFIZER ROCHE. The other authors declare that they have no competing interests.

Additional information

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