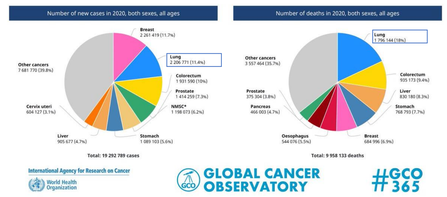
Monitoring *GALNT13* in blood of non–small cell lung cancer patients with serial evaluation using digital RT-PCR. A potential new liquid biopsy biomarker.

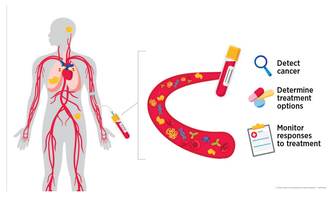
Eugenia Fernández[1,2], Diego Santana[1], Mariana Carrasco[1], Marcos Acosta[1], Diego Touya[1], Nabila Elgul[4] Eduardo Osinaga[1, 2, 3] and Nora Berois[1,2]

1- Servicio de Oncología, Laboratorio de Oncología Molecular, Hospital Maciel, ASSE, Montevideo, Uruguay
2- Laboratorio de Glicobiología e Inmunología Tumoral, Institut Pasteur de Montevideo, Uruguay
3- Departamento de Inmunobiología, Facultad de Medicina, Udelar, Montevideo, Uruguay
4- CASMU, Centro Asistencial del Sindicato Médico del Uruguay, Montevideo.

Background

Lung cancer is the second tumor with the highest incidence and the leading cause of death for both sexes[1]. Most cases present at advanced disease, where tissue biopsies are often inaccessible or do not have enough material for molecular analysis. Thus, liquid biopsies appear as powerful tools to non-invasively analyze lung cancer phenotype and progression as well as drug resistance[2,3]. Protein *O*-glycosylation abnormalities are found in most carcinomas. Several clinically useful biomarkers detecting these changes have been developed. The initial step of synthesis of mucin-type *O*-glycans is catalyzed by a complex enzyme family (UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferases; GalNAc-Ts). We previously found that GalNAc-T13 isoenzyme is a strong predictor of poor clinical outcomes in neuroblastoma[4] and breast cancer patients[5]. As shown in ISLB congress 2023, given that GalNAc-T13 is also expressed in non-small cell lung cancer (NSCLC)[6], we evaluated *GALNT13* expression by nested RT-PCR in blood samples of 119 NSCLC patients from Maciel Hospital, Montevideo, finding a possible correlation with clinical outcomes. In this work, we quantified *GALNT13* expression by RT-Digital-PCR in serial samples of peripheral blood of NSCLC patients, to evaluate this enzyme as a new potential liquid biopsy biomarker useful in patients' follow-up.





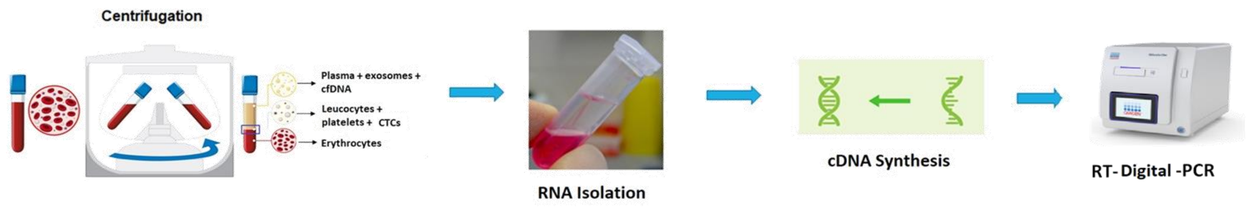


Figure 1: Liquid Biopsy Protocol. Analysis of *GALNT13* expression in peripheral blood of NSCLC patients by RT- Digital -PCR

Methods

The specificity of the method was evaluated by analyzing 48 peripheral blood samples from healthy individuals (Servicio Hemoterapia, Hospital Maciel, Montevideo); while sensitivity was evaluated using a dilution model with cell lines that simulate the clinical sample to be studied. *GALNT13* expression was evaluated by RT-Digital-PCR in peripheral blood of 39 NSCLC patients from Maciel Hospital, Montevideo. Results were correlated with clinical-pathologic data, treatment response and patients' survival.

Results

Cut-off was defined by analyzing *GALNT13* expression in 48 healthy individuals. 39 NSCLC patients with 1-9 follow-up samples were studied. The median age was 64 (ranging from 44 to 78); 56.4% corresponded to males and 43.6% to females. *GALNT13* expression was detected in 64% of NSCLC patients. Concerning histological types, *GALNT13* was expressed in 56% of adenocarcinoma patients, 75% of squamous cell carcinoma patients, and 83% of other histologic types. Relating to stages, the enzyme was found in 28.6% of stage I/II patients, 73.3% of stage III, and 73.7% of stage IV patients (Table 1). Similarly, as seen by NESTED PCR, *GALNT13* expression in the initial samples was significantly correlated with clinical outcomes (Figure 2). When analyzing serial samples, we noticed that in those patients in remission, the expression of the enzyme decreased significantly. In contrast, patients experiencing disease progression showed elevated *GALNT13* expression, sometimes even prior to imaging detection (Figures 3 and 4).

		GALNT13 (+) n (%)	GALNT13 (-) n (%)
Patients	**n = 39**	**25 (64)**	**14(36)**
Age	**Median: 64 Range: 44-78**		
Sex	**F: n=18 (46%) M: n=21 (64%)**		
Histology	**ADC: n=25 (64%)**	**14 (56)**	**11 (44)**
	SCC: n=8 (21%)	**6 (75)**	**2 (25)**
	Other: n=6 (15%)	**5 (83)**	**1(17)**
Stage	**I-IIIA: n=14**	**5 (36.7)**	**9 (64.3)**
	IIIB-IV: n=25	**20 (80)**	**5 (20)**

Table 1: *GALNT13* expression in NSCLC patients

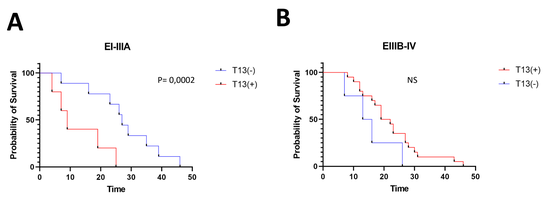


Figure 2: Kaplan–Meier overall survival rates related to *GALNT13* expression. (**A**) Early-stage tumors (stages I, II and IIIA) (n = 14). (**B**) Advanced stage tumors (stages IIIB, IIIC and IV) (n = 25).

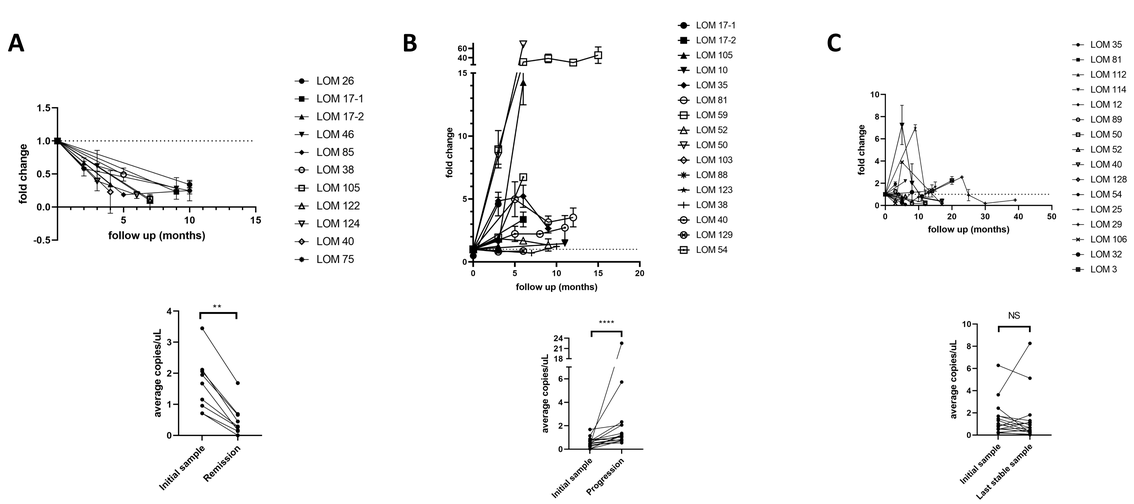


Figure 3: *GALNT13* expression in NSCLC patients through the disease. Patients in remission (A), Progression (B) or stable disease (C). Mann Witney test. Ns P > 0.05 ; ** P ≤ 0.01 ; **** P ≤ 0.0001

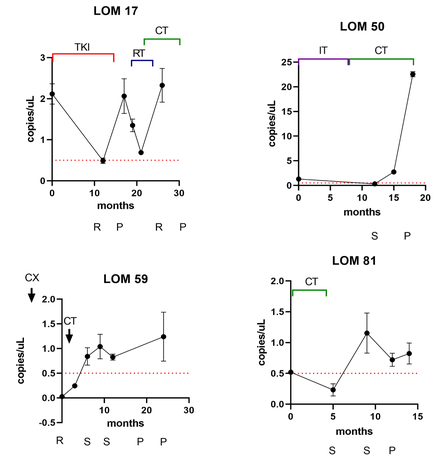


Figure 4: *GALNT13* expression in NSCLC patients through the disease- Examples of individual patients. TKI- Tyrosine Kinase Inhibitor. RT- Radiotherapy. CT- Chemotherapy. IT- Immunotherapy. R – Regression. P – Progression. S- Stable disease

Conclusions and Perspectives

In this study, we suggest that monitoring *GALNT13* expression in blood of non–small cell lung cancer by digital RT-PCR could be a potential new follow-up biomarker in liquid biopsy for lung cancer patients. Further work is needed to confirm our findings. We are now working on the characterization of *GALNT13* in peripheral blood, in other to confirm if the expression of this enzyme is restricted to circulating tumor cells (CTCs) and broaden our knowledge of possible molecular pathways implicated.

References: 1- Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021;71(3):209-249. doi:10.3322/caac.21660; 2- National Cancer Institute. Bethesda M. SEER Cancer Stat Facts: Lung and Bronchus Cancer. https://seer.cancer.gov/statfacts/html/lungb.html. ;3- Di Capua D, Bracken-Clarke D, Ronan K, Baird AM, Finn S. The Liquid Biopsy for Lung Cancer: State of the Art, Limitations and Future Developments. Cancers (Basel). 2021;13(16):3923. doi: 10.3390/cancers13163923. ; 4- Berois N. et al. ppGalNAc-T13: a new molecular marker of bone marrow involvement in neuroblastoma. Clin Chem. 2006; 52:1701-12. doi:10.1373/clinchem.2006.067975. ; 5. Fernandez E. et al. Polypeptide-GalNAc-Transferase-13 Shows Prognostic Impact in Breast Cancer. Cancers (Basel). 2021; 13(22):5616. doi:10.3390/cancers13225616. ; 6. Berois N. et al. Polypeptide GalNAc-transferase-13 shows independent prognostic impact in adenocarcinoma lung cancer patients treated with neoadjuvant chemotherapy. J Glycobiol 2017, 6:2(Suppl) doi:10.4172/2168-958X-C1-009