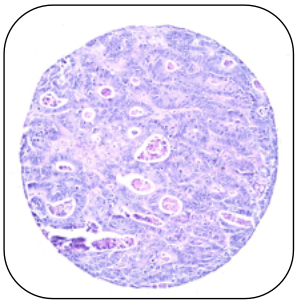
Montreal, March 21, 2018

Dear IHPBA Research Committee,

My sincerest gratitude to **IHPBA** which, with the support of the **CGF E Alexander Stent Fund, Bank of America, N.A., Trustee**, awarded me the 2017-2018 **Kenneth Warren Fellowship Grant** to support my research project that focuses on the **immunosuppressive impact of the adenosine pathway in colorectal liver metastasis**. A study I am working on under the mentorship of **Dr. Simon Turcotte** at the Research Center of the **Centre Hospitalier de l'Université de Montréal** (CRCHUM).

Cancer immunotherapy using antibodies to block immune checkpoints is a major breakthrough in oncology, but colorectal cancer thus far appears resistant to current approaches. New immune checkpoints linked to immunosuppressive pathways at play within the tumor microenvironment are being elucidated. As such, **extracellular adenosine and CD73 represent promising targets in immuno-oncology**. Extracellular adenosine is a metabolite of ATP released by cancer and other cells in the tumor microenvironment. Adenosine mediates its immunosuppressive effects notably by binding to the A2A receptors expressed by activated T cells and natural killer cells. CD73 is the rate-limiting enzyme in the ATP to adenosine degradation pathway. In addition to its immune suppressive effect, CD73 is also an adhesive and signaling molecule that mediates cancer invasion, neovascularization and metastasis.

In this study, we hypothesize that **high CD73 expression** in colorectal cancer liver metastases (**CRCLM**) can define a subgroup of metastases with distinct immune features in patients with **poorer prognosis**. This groundwork will define the biological relevance and potential rationale of targeting CD73 in patients with CRCLM.



From our prospectively maintained database, we identified a cohort of 234 patients who underwent CRCLM resection with curative intent at our institution and retrieved their clinicopathological data. After pathological review, **tissue microarrays** (TMA) of 401 CRCLMs were built to perform high throughput **immune phenotyping**, using 6 intratumoral cores per CRCLMs, of up to 3 CRCLMs per patient. We next performed a **multiplex immunofluorescence** on the TMAs to concurrently quantify CD73 expression, cytokeratins (CK8/18, to differentiate stromal vs. epithelial expression patterns), and DAPI to stain the nucleus of live cells (see Figure).

Using this methodology, we found interesting associations between **high CD73 expression and adverse clinopatholological features, as well as worse outcome in CRLM patients.** Study results we are keen on publishing shortly.

The next aim of the project is to **test whether serum levels of circulating soluble CD73 correlates with intra-tumoral CD73 expression**. Serum derived from pre-operative blood draws, available for >90% of patients in this cohort, will be thawed and tested in triplicates for the concentration of soluble CD73 by ELISA. The values obtained will be correlated with intra-tumoral CD73 expression and will determine whether circulating soluble CD73 could serve as a biomarker of intra-tumoral CD73. This would represent a **non-invasive, "liquid biopsy" tool** for patient stratification and selection. Looking forward to present these (and future) results at the **14th IHPBA World Congress in Melbourne Australia.**

Kind regards,

Nouredin MESSAOUDI, MD