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Perspective on the treatment of non-small cell lung cancer in the context of potential SARS-CoV-2 infection during the pandemic

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Abstract

SARS-CoV-2 infections are rising at an alarming rate and various aspects of this pandemic must be quickly and adequately addressed in order to enhance effective healthcare delivery and protect at risk populations such as cancer patients. Preventing Covid-19 infection must be a top system wide priority to avoid mortality, and considerable financial and disease burden. Most cancer patients, and in particular those with tumors resistant to chemotherapy are particularly vulnerable to infection. In this review, we connect potential viral infection of patients with lung tumors that have somewhat quiesced the immune response in the tumor microenvironment and categorize target molecules in metabolism that may be used to identify at risk patients leading to more effective treatment regimens; keeping continuity of therapy and disease prevention during a very tumultuous period of time surrounding the pandemic.

Keywords: *immunosuppressive; infection; lung cancer; metabolism; SARS-CoV-2; treatment; tumor microenvironment.*

1. Introduction

Cancer patients are considered highly vulnerable to SARS-CoV-2 infection, with inconsistent and scattered data reports coming in weekly to describe the pandemic. Much is unknown at this time regarding the clinical characteristics of SARS-CoV-2 infected cancer patients, and especially patients resistant to chemotherapy, but airway mediated infection is widely accepted. A small retrospective study of patients from Wuhan, China in January-February 2020 recently noted that lung cancer was the most frequent cancer type (25.0%) among patients with laboratory confirmation of infection (Zhang et al., 2020). A total 53.6% of the patients studied had severe events and a mortality rate of 28.6%. More alarming was the report in this study that if patients received their last antitumor treatment within 14 days, the risk of developing severe events increased significantly [hazard ratio (HR) = 4.079, 95% confidence interval (CI) 1.086-15.322, P = 0.037] (Zhang et al., 2020). These observations highlight the importance of timely identification of tumor status among patients who appear for COVID-19 testing. In fact, the authors recommend that cancer patients should avoid treatments causing immunosuppression or have their dosages decreased in case of SARS-CoV-2 coinfection (Zhang et al., 2020). Since the time of this study, the number of infected individuals in Europe and the Americas has skyrocketed, creating a more serious and complicated situation for the treatment of lung cancer patients who are or could be positive for infection.

Lung cancer is the most common cause of tumor related death in the world (Miller et al., 2016). Surgery is the current treatment for early stage lung cancer but most patients already have locally advanced or metastatic disease at the time of

diagnosis, hence many are potentially immune deficient. Cisplatin-based chemotherapy combined with radiation therapy or chemotherapy alone remains the primary modality of treatment for stage 3 and 4 disease. New forms of cancer therapy which use the body's own immune system to fight cancer have emerged lately, in particular, check point inhibitor(s) such as nivolumab and pembrolizumab. While these drugs offer a longer duration of response compared to other 2L chemotherapy, the response rate is low (15-20%). Thus, improvement of therapeutic efficacy is needed for 2L treatment by understanding the complex molecular changes that may confer resistance in cisplatin resistant lung cancer (refer as **CR**) cells. Our laboratory has found that increasing ROS (reactive oxygen species) level is a specific and yet common feature found in CR cells. Through our research we have shown that manipulation of ROS levels selectively killed CR cells. Moreover, we have found the underlying mechanism for increasing ROS and its relationship with metabolic reprogramming and immune evasion. These novel findings can lead to new care plans for these patients by targeting tumor metabolism.

We have discovered a common biochemical alteration in all CR cells, wherein high basal level of ROS and increased secretion of thioredoxin-1 (TRX1; an antioxidant) levels are the significant modulators in the metabolic reprogramming seen in these cells (Wangpaichitr et al., 2012; Wangpaichitr, Wu, Li, Nguyen, Kandemir, et al., 2017). In addition, recent reports as well as established research, into viral infection have demonstrated elicitation of immunological reactions, cytokine release and immune-cell infiltration producing various reactive oxygen species (ROS) (J. Z. Wang, Zhang, & Bai, 2020). In connection to these findings, we recently uncovered another unique resistance mechanism where CR cells activate the kynurenine pathway (L-tryptophan catabolism) when they encounter excessive oxidative stress in order to maintain a finite level of ROS for their growth and proliferation. Hence, this pathway becomes an essential pathway for their survival. Importantly, L-kynurenine (KYN), a product of the kynurenine pathway (KP), plays a key role in reprogramming naïve T-cells to the immune suppressive regulatory T-cell (T-reg) phenotype (Figure 1)

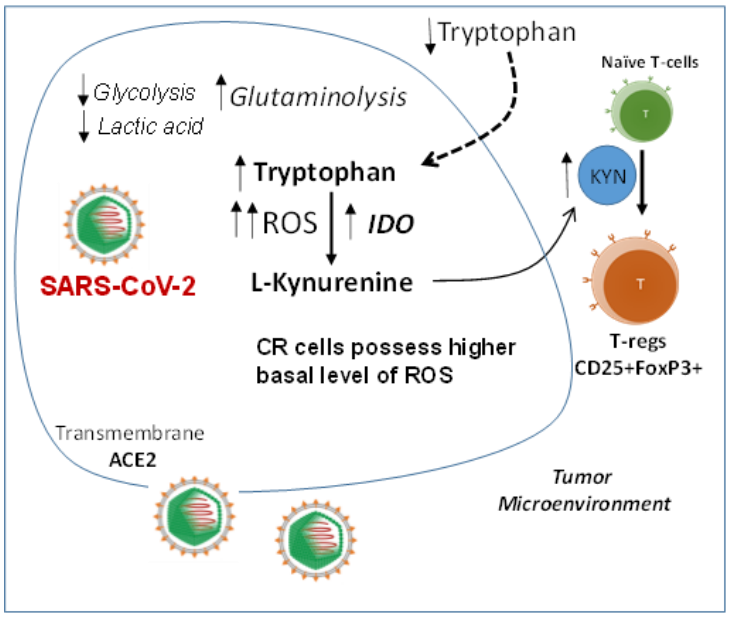

Figure 1 CR cells do not primarily utilize glucose but rather consume amino acids such as glutamine and tryptophan for survival. This metabolic switch is due to increased ROS production hyperactivating KP to balance oxidative stress and maintain cellular growth and proliferation. KYN is oxidized through indoleamine 2,3-dioxygenase (IDO) and plays a key role in reprogramming naïve T cells to the immune-suppressive Treg phenotype. ROS is further increase by SARS-CoV- 2 infection which in turn creating more immune suppressive microenvironment.

The expression of angiotensin-converting enzyme 2 (ACE2) protein has been observed in numerous cancer cells, we believe that this finding demonstrates the potential vulnerability to SARS-CoV-2 infection by tumors resistant to cisplatin-based chemotherapy (Figure 2). With this we believe that shielding of lung cancer from immune surveillance, resulting from metabolic switching which hyper-activates the KP, increases the sensitivity of these patients to SARS-CoV-2 infection in the lung. Importantly, we believe that various tumor metabolic characteristics may be applied to screen for patients susceptible to SARS-CoV-2 infection. Hence, in the changing environment of the pandemic, treatment outcomes may be improved for cancer treatment efficacy as well as avoidance of infection. Overall, recognizing the interrelationship between cellular metabolism and immune response will be critical in the development of better therapeutic plans for patients at risk for SARS-COV-2 infection, and further resistance to cancer chemotherapy.

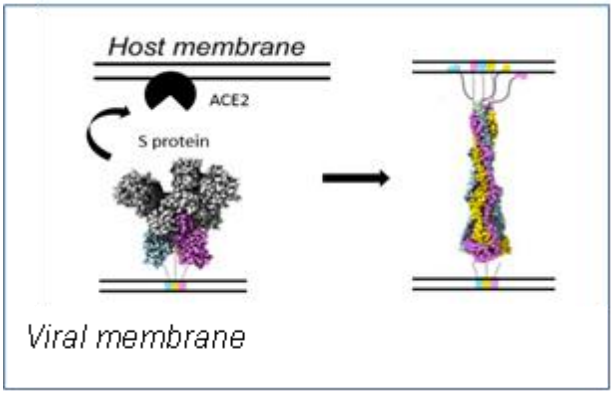

Figure 2 Binding scheme of SARS-CoV-2 to host. Binding of Spike protein to the ACE2 results in viral-host membrane fusion and the entrance of virus into host cell.

2. Significance for patient care

Covid-19/ SARS-CoV-2 airway mediated infection has increased lethality in immunocompromised patients, and many US hospitals are being flooded positive inpatients at this time. This surge in necessary, immediate, and significant patient care is pushing various hospital systems and healthcare organizations, as well as the public sector to their maximum capacity. Lung cancer is the most common cancer in various US healthcare systems and leading cause of cancer deaths nationally. Many of these patients have had to receive necessary treatments in 2020 during the pandemic. Recent clinical reports from China show that lung cancer patients have significantly worse outcomes for morbidity and mortality associated with COVID 19 and that many of those patients are men. Many lung tumors resist cisplatin, (first line standard of care), and we have published widely that resistance is based on metabolic alterations that suppress T-cells activation in the tumor microenvironment, resulting in local immune-deficient tissues. Hence, resistant patients must rely on 2nd and 3rd line therapies, further prolonging an immune-compromised state.

Currently, the impact of tumor metabolism on the tumor microenvironment is not well established and any correlation of SARS-COV-2 infection is unknown. Our previous and present data have shown that CR cells secrete TRX1 and up-regulate the KP, respectively. Interestingly, high TRX1 and KYN levels in the microenvironment can enhance regulatory T-cell (T-reg) infiltration and stimulate the conversion of naive T-cells to T-reg (de Araujo et al., 2017; Platten, von Knebel Doeberitz, Oezen, Wick, & Ochs, 2014; X. Wang, Dong, Li, Li, & Hong, 2015), creating an immunosuppressive environment. Furthermore, we found that CR tumors utilized oxidative metabolism (OXMET) and had highly increased amino acid uptake (Wangpaichitr, Wu, Li, Nguyen, Shah, et al., 2017). Consequently, this

microenvironment is deprived of amino acids, thus creating an additional unfavorable condition for the viability of cytotoxic effector T-cells which are highly anabolic and require high amounts of amino acids for growth (Fox, Hammerman, & Thompson, 2005; Jones & Thompson, 2007). These factors therefore contribute to a unique microenvironment which favors the presence of T-reg rather than cytotoxic T cells, making that tissue vulnerable to infection. Overall, future investigations of tumor cells and their microenvironment, and the targeting of binding of SARS-CoV-2 to ACE2, could ultimately identify a population of lung cancer patients who will have improved outcomes due to diligent screening and informed treatment strategy.

3. SARS-CoV-2 infection

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel coronavirus with significant human-to-human pathogenesis, where pulmonary infection is key, resulting in the declaration of a global pandemic. In reports from the last couple of months, resulting disease required hospital admission in 20% of cases, with some patients requiring care in Intensive Care Units (6%), intubation (2.3%), or resulting in death (Guan et al., 2020). The rate of death in different populations has ranged from 1.4- 2.4% in China to 7.2% in Italy (Guan et al., 2020; Livingston & Bucher, 2020). Risk of death increases with age, and is highest among those who have co-morbidity or are immunocompromised (Guan et al., 2020; Wu et al., 2020). Of particular importance for our work is the report that in China hospitalized cancer patients had a high frequency (39%) of ventilation or death compared to patients without cancer (8%) (Liang et al., 2020). This finding demonstrates that cancer patients are at much greater risk for worst outcomes than patients without cancer. Pulmonary and systemic clinical pathogenic analysis from autopsy data, and clinical cases from patients hospitalized in ICU show increased levels of plasma inflammatory cytokines, neutrophilic infiltration, macrophages, monocytes, and minimal lymphocytes with mostly CD4+ T-cells, immune deficiency, and rising lactate dehydrogenase (Guan et al., 2020; Huang et al., 2020; Wu et al., 2020). Hence CD4+ T cells are found in the reported clinical data thus far, and infection causes various metabolic and immune modulations whose effects are not clear at this point.

Some studies have reported a differential decline in CD4 and CD8, and increased programmed cell death protein 1 [PD1], cytotoxic T-lymphocyte-associated protein 4 [CTLA4], and decreased intracellular gamma-interferon) and inflammatory cytokines (Qin et al., 2020). Due to the fast pace of the spread over the last 4-5 months, there is a lack of studies characterizing a specific immune response in patients infected with SARS-CoV-2, and no studies among cancer patients with analysis of significant molecular signaling. Immune suppression and other comorbidity predict higher morbidity and mortality with SARS-CoV-2 infection. Patients who recovered from COVID-19 syndrome have evidence of humoral neutralizing antibodies (Walls et al., 2020) and SARS-CoV-2 specific T-cells, again showing the potential role of T-cells reported thus far is characterizing the immune responses against this virus. We have not found reports describing the specific differences or similarities between lung cancer patients and patients with previously healthy lungs, and the heightened risk for infection and death due to possible changes in expression in PD-1/CTLA4 (immune checkpoint inhibitors), or T cell deficiency in the infected tissue. Hence, an additional gap exists that must be addressed via the evaluation of SARS-CoV-2 related signaling in lung cancer in order to guide future care plans and screening strategies.

4. Propensity for infection in the lung

Binding of the SARS-CoV-2 to cell membranes is crucial to the infection and replication process since both SARS- CoV and SARS- CoV- 2 share ACE2 as the key cell surface receptor, and TMPRSS2 as the major protease facilitating their entry into host cells (Hoffmann et al., 2020; Walls et al., 2020; R. Yan et al., 2020) (Figure 2). Based on expression analysis from 12 healthy donors, lung tissue expresses ACE 2 (Lukassen et al., 2020) distributed across cell types in lung tissue, indicating that the lung could be infected broadly. Blocking of this virus-ACE2 binding, could prevent the fusion to the host membrane. In order to investigate the expression of ACE2 in lung cancers, ACE2 protein could be detected in

different tumor cell types with variable resistance to chemotherapy. Hence, any tumors that express ACE2 would be at increased risk for infection by SARS-Cov2 via the receptor. We consider this concept is particularly relevant to resistant tumors since the patients will ultimately have to be exposed to numerous lines of therapy, also potentially affecting their immune competency. The expression of ACE2 in tumor cells, along with the alarming results of SARS-Cov2 infection described in China, lead us to promote the need for additional research into pre-existing or altered T-cell responses in tumors affected by mechanisms of resistance which alter tumor sensitization to the immune response and other therapies (Trujillo, Sweis, Bao, & Luke, 2018).

In this environment, infection as well as cancer proliferation may occur in an unchecked fashion. Although T-reg induction is complex, it is likely that the tumor microenvironment of NSCLC is responsible for their residence in the tumor. T-regs are characterized by their ability to suppress immune responses to allogeneic antigens, to anti CD3 and presumably to tumor antigens (Shevach, 2002). It appears extremely likely that tumor residing T-regs contribute to the lack of a spontaneous immune response to the tumor in NSCLC patients. Moreover, NSCLC tumors are known to produce IL-10, TGF-β and PGE-2 (Hidalgo, Zhong, Doherty, & Hirschowitz, 2002; Kamiya et al., 2003; Neuner et al., 2002; Saji et al., 2003) and are infiltrated by T-reg that actively suppress T-cell priming (Woo et al., 2002). We have previously reported that CR cells secrete less lactate (Wangpaichitr et al., 2012; Wangpaichitr, Wu, Li, Nguyen, Kandemir, et al., 2017) which may very well impact the fate of surrounding T-cells as well. Lactic acid can be taken up by neighbor cells, including immune cells, to perpetuate the glycolytic pathway and upregulate HIF1α (Cascone et al., 2018; Sonveaux et al., 2008). In fact, effector T-cells (T-eff) rely on glycolysis to expand and this growth is greatly dependent on HIF1α (Doedens et al., 2013). A diminished amount of lactate may prevent a robust immune response to the neighboring tumor. Furthermore, mice deficient in HIF1α fail to mount a strong T-cell response and have increased T-reg populations (Dang et al., 2011; L. Z. Shi et al., 2011). All of these findings support a concept wherein the metabolic modifications in CR tumors impact immune cells in the tumor microenvironment. We believe that the examination of modeled SARS-CoV-2 infection will demonstrate the propensity for infection when challenged with virus in SA1.

Additional existing supporting clinical evidence for this concept is the finding that immune checkpoint inhibitors are more effective in patients who fail chemotherapy (primarily platinum containing regimens) (Rizvi et al., 2015; Wangpaichitr, Kandemir, et al., 2017; F. Yan et al., 2016). Hence, the possibility that platinum resistant tumors are actually enriched with immunosuppressive T-reg cells is likely. We previously dissected the underlying mechanism(s) that prevent the detection and potential elimination of CR tumors by immune surveillance and consequent immune response. Cisplatin sensitive (LLC) and resistant (LLC-CR) lung tumor cells were allografted into and we found lineage-specific marker of T-reg cells were higher in LLC-CR when compared to parental counterpart which further indicated the involvement of KP in CR tumors. Furthermore, higher expression of TGFβ, a well-known immunosuppressive cytokine that is released by T-reg (M. L. Chen et al., 2005) and has been shown to activate indoleamine 2,3-dioxygenase 1 (IDO1) biosynthesis (Mbongue et al., 2015; Y. Shi & Massague, 2003). In fact IDO1 expression is most likely elevated allog in resistant tumors compared to sensitive tumors. We believe that molecular alterations found in CR cells can create a highly immunosuppressive environment which favors the growth of tumors and the potential for infection by viruses such as SARS-CoV-2.

The forkhead family transcriptional regulator FoxP3 is also another lineage-specific marker of T-reg cells. Mice deficient in FoxP3 display a total lack of T-reg cell populations (Fontenot, Gavin, & Rudensky, 2003), and overexpressing FoxP3 can lead to the conversion of effector T-cells into T-reg cells (Hori, Nomura, & Sakaguchi, 2003). Thus, there is no doubt that FoxP3 is a marker of T-reg and that it is expressed in the immune suppressive cells. Interestingly, many reports have recently shown that that FoxP3 mRNA as well as protein can be detected in various cancer types including lung cancer (G. Y. Chen et al., 2008; Karanikas et al.,

2008). CR NSCLC tumors that express FoxP3 compared to sensitive cells could be an additional indicator of further promotion of a deficient immune environment susceptible to cancer growth and infection.

5. Metabolic alterations in CR tumors involve the contribution of the kynurenine pathway (KP)

While L-tryptophan (TRP) is an essential amino acid, required for protein synthesis, the catabolism of TRP generates KYN via the KP. It is responsible for the catabolism of approximately 99% of ingested TRP not used for protein synthesis (Peters, 1991). At the first step, TRP is oxidized through indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) to form formylkynurenine which is later degraded to KYN (Figure.1). TDO is primarily present in the liver and is not expressed in lung tissue or lung tumor (van Baren & Van den Eynde, 2015). An increased ratio of KYN/TRP can serve as an indicator of TRP degradation (Creelan et al., 2013; Suzuki et al., 2010). We have found that uptake of TRP is significantly increased in CR cells.

We have previously shown that all our CR cells possess higher ROS as well as superoxide dismutase (SOD) expression and activities (Wangpaichitr et al., 2009). As a result, CR cells can utilize superoxide as substrate to balance ROS. Similar to SOD, IDO is a unique enzyme that can exploit superoxide as a substrate (Davis & Liu, 2015). Thus, high ROS in CR cells significantly boosts IDO activity. Indeed, tumors of resistant cells showed increased IDO expression levels in vivo. Hence, these findings suggest that when cancer cells acquire greater resistance to cisplatin, ROS production also progressively increases.

We reviewed a large publicly available database of lung cancer patients for survival data of those with high expression of IDO since it is a suggested predictor for poor prognosis (Liu et al., 2016; Schalper et al., 2017; Theate et al., 2015) and is often found in late stage lung cancer. Although we did not gather treatment history for these patients, and no study had been reported in CR tumors, the KM plot showed a lowered survival probability for patients with high IDO expression. Importantly, using IDO expression as a biomarker still remains controversial since studies have shown no significant correlation between IDO expression and clinic-pathological parameters (Ferdinande et al., 2012; Gao et al., 2009; Karanikas et al., 2007). But there are tumor and tissue specific parameters that will need to be evaluated in order to provide additional insights. All of the data support molecular expression in resistant lung tumors promoting the T-reg phenotype and a lowered localized immune response.

6. Kynurenine/ROS axis and SAR-CoV-2

The role of ROS in SAR-CoV-2 remains elusive; however, it has been shown that virus infection disrupts the redox balance by decreasing glutathione (antioxidant) production and promoting the propagation of the virus progeny, thus resulting in cell death (26761025). Moreover, it is known that influenza virus infection can induce expression of interferons (IFNs), which upregulated production of IDO1. Reports have shown that KYN biosynthesis was activated in macrophages in response to virus stimuli (ie. herpes simplex virus 1 and 2) (PMID27960276). This activation may affect the expression of tumor molecules, as well as impact the tumor microenvironment. Recent discoveries revealed that KYN can serve as an endogenous ligand for the aryl hydrocarbon receptor (AhR) in cancer cells (29). KYN forms a complex with AHR and is translocated into the nucleus to modulate target genes as a ligand-activated transcription factor (30). Importantly, AhR activation during virus infection disrupts host immunity and causes increased lung inflammation and mortality in mice (pmdi**25283860)**. Hence, receptor activation and/or expression along with changes in KYN expression may create unique signaling effects among infected lung cancer patients. The level of virus-induced IFN is also increased in AhR-deficient cells and mice, thus leading to the suppression of viral replication (**19027719).** We have shown that AhR antagonists, such as CH-223191 suppress IDO1 activity, whereas addition of KYN increased IDO1 activity (REF). It is noteworthy that activation of IDO1 enzymatic activity requires ROS as a co-factor (REF).

AhR antagonist therapeutic efficacy against virus-infection remains unclear due to that fact that AhR responds differentially to diverse intrinsic and extrinsic ligands and affects

multiple types of immune cells. We firmly believe that regulation of ROS and the kynurenine pathway could be influenced by interactions with viral pathogens and should be considered during development of novel antiviral drugs and therapies.

7. Conclusion

While immunotherapy with check point inhibitors (PD-1) has been approved for the second line treatment of NSCLC (the majority of these patients have failed platinum containing regimens), the response rate remains low (15-20%) but the duration of response is long. Thus, there are a large number of patients who require other therapy and may be more at risk for SARS- CoV- 2 infection and subsequent severe disease. In addition, to our knowledge there are no studies dissecting the molecular changes occurring in tumor metabolisms. In our previous work, we have shown that CR cells and patients have high levels of ROS and that KP is highly activated in CR cells when compared to parental counterparts or normal cells. KP utilizes and regulates ROS, thus this pathway became essential for CR's cells survival and evasion from immune surveillance by promoting T-reg differentiation.

Future studies must evaluate the key metabolic and immune profiles altered by SARS-CoV-2 infection in lung tumors sensitive and resistant to CP chemotherapy. Infection by SARS-CoV-2 will produce numerous unknown molecular alterations in normal cells, and lung cancer cells that will vary based on metabolic necessities and the localized immune environment. We have reported that CR lung cancer cells possess higher IDO1 activity resulting in greater KYN production reprogramming naïve T-cells to the immune suppressive regulatory T-reg phenotype (Walls et al., 2020; Hoffmann et al., 2020). This creates locally immunocompromised tissue. It is not known if additional changes to metabolism will occur in tumor cells stressed by virus infection. We anticipate that SARS-CoV-2 mediated signaling will further enhance the T-reg population leading to a more immunosuppressive microenvironment.

We have reported that CR cells possessed increased numbers of mitochondria and consumed more oxygen, resulting in significantly higher basal levels of ROS (Wangpaichitr, Wu, Li, Nguyen, Shah, et al., 2017). In addition, we reported that CR tumors do not follow classic aerobic glycolysis (Warburg effect (Warburg, 1956a, 1956b)) by switching to oxidative metabolism (OXMET). These metabolic derangements led us to the discovery that L-tryptophan catabolism (kynurenine pathway; KP) is also upregulated in resistant cells. Significantly, KP is one of the key mechanisms in achieving peripheral tolerance and evading immune surveillance in tumor cells (Beatty & Gladney, 2015; Heng et al., 2016; Moon, Hajjar, Hwu, & Naing, 2015; Platten et al., 2014) (not exposed to virus), and we believe that this resultant immune deficiency increases the possibility for SARS-CoV-2 infection in the lung and systemic spread. Additional studies should identify the immune-sensitivity of CR tumors to SARS-CoV-2 infection and rely on the rationale of comparing normal and lung tumor models by providing a framework for the study of outcomes with potential SARS-CoV-2 infection. Moreover, we firmly believe that a cancer treatment based on blocking the interaction between the virus and ACE2 may demonstrate improved outcomes in SARS-CoV-2 infection.

8. DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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