The Effects of EGFR Mutations in Tumors on the Outcome of an Abscopal Effect

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Abstract

Lung cancer is the leading cause of cancer related death in Canada and worldwide.[1,2] One treatment method against lung cancer is a type of immunotherapy (IT) which targets mutated epidermal growth factor receptor (EGFR.) A combination of IT and radiotherapy can cause the abscopal effect, or localized treatment causing system wide results.[3] However, the mechanisms of the abscopal effect are not fully understood. This study aims to determine whether the presence of an EGFR mutation impacts the outcome of the abscopal effect. Data on 339 lung cancer patients from across Alberta participating in a different study was compiled from provincial oncological records (ARIA-MO) and recorded onto an EXCEL spreadsheet. Data added to the previous work included the dates of discovery for brain, liver, bone, and other metastases. Patients with EGFR mutations were found to have, on average, a lower OS while also having increased rates of incidence of brain, bone, and liver metastases. As such, patients with EGFR mutations were shown to be less likely to experience the abscopal effect. This increased understanding of the abscopal effect and what factors might lead to it is key to increasing the quality of life and life expectancy for those with cancer.

Introduction

Lung cancer is the leading cause of cancer-related death in Canada, with a 17% likelihood of living five years or longer compared with the average person without cancer.[2] On a larger scale, lung cancer is the leading cause of cancer-related deaths worldwide.[2] Non-small cell lung cancer (NSCLC), any type of lung cancer that is not small cell lung cancer (which is characterized by

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small cells, not a lot of cytoplasm, and missing nuclei[5]), makes up 85% of lung cancers.[6] It is mostly linked to smoking as a cause, but non-smokers and infrequent smokers can develop this type of cancer as well. There are three main types of NSCLC: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

Treatment options traditionally included chemotherapy (CT) and radiotherapy (RT), but due to insensitivity to RT and CT[4], poor 5-year survival rates, and increases in the understanding of immunity and NSCLC[7], immunotherapy (IT) has become an increasingly viable treatment method. IT is the utilization of the body's own immune system to target and kill cancer cells.[8] One commonly used type of IT is the use of immune checkpoint inhibitors, blocking receptors such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death-ligand 1 (PD-L1) to enable an immune response.[9] These receptors appear on either immune cells or cancer cells to prevent the immune system from attacking the body's own cells, an ability that often hides cancer cells within the body. Drugs like pembrolizumab, nivolumab, and ipilimumab bind with these receptors on the surface of either the tumor cell or the immune cell, stopping the two cells from binding together.[10] This prevents the immune system from recognizing the mutated cancer cells as part of the body and allows it to instead recognize them as something foreign, initiating an immune response.

Another type of IT works through targeting oncogenic genes, like the genes coding for the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK). Almost 20% of lung cancers carry mutations in these genes.[11] They both produce proteins that appear on the surface of cells to bind with corresponding proteins on other cells to initiate mitosis. When mutated, these genes cause the unchecked cell division characteristic of cancer.[11] The EGFR mutation specifically is more common in patients with adenocarcinoma.[12] Treatment targeting this mutated protein include erlotinib, gefitinib, and panitumumab.[13] These drugs work similarly to immune checkpoint inhibitors in that they bind with the receptors in the cell membrane to prevent cells from binding

one another, stopping them from duplicating.[14] This means that the tumour cannot grow any bigger, giving the immune system an advantage in destroying the tumour. Despite these advancements, response rates to immunotherapy are either very low, with about 13% of those treated with immune checkpoint inhibitors responding[15], or patients quickly become immune to it. Treatments utilizing the presence of an EGFR mutation quickly loose their efficacy due to the cancer developing resistance.[16] As such, researchers have been looking into ways to boost or improve the results of these treatments in patients.

One such method is the initiation of the abscopal effect, the ability of localized treatment to produce system-wide results.[17] Initial case reports were recorded in the early 70s, yet it is still not fully understood.[3] It is known that when local RT induces cell death, the cell releases immunogenic factors such as danger-associated molecular patterns (DAMPs) and tumour-associated antigens (TAAs).[3] The release of these neoantigens, antigens the body has never been exposed to before, triggers a tumour-specific immune response. However, immune-suppressant effects of the tumour prevents the abscopal effect from triggering consistently. Today, it has been widely accepted that IT combined with RT boosts the chances of an abscopal effect occurring, but an optimal treatment regimen that produces this effect to its fullest extent is still unknown.

This study aims to shed light on whether or not a patient with non-small cell lung cancer, treated with immunotherapy, and with EGFR oncogenic mutations present in the tumour, will have the outcome of an abscopal effect influenced by the presence of this mutation.

Scientific Question

If a patient with non-small cell lung cancer, treated with immunotherapy, has EGFR oncogenic mutations present in the tumour, will the outcome of an abscopal effect be influenced by the presence of this mutation?

Hypothesis

If a patient with non-small cell lung cancer, treated with immunotherapy, has EGFR oncogenic mutations present in the tumour, then they will be less likely to have an abscopal effect occur. This is because those with the EGFR mutation are more likely to have reduced survival[18], lymph node metastasis, and insensitivity to chemotherapy.[19] Additionally, any benefit to prognosis received from EGFR specific immunotherapy treatments is quickly lost due to how quickly EGFR-mutated tumors gain resistance.[16]

Methodology

Variables

The independent variable in this study is whether or not the EGFR oncogenic mutation is present. In response, the dependent variable would be the outcome of an abscopal affect. Measuring this outcome is not easy to determine, so analyzing time to recurrence and/or progression will be utilized to define whether or not an abscopal effect occurred. The control variables include where the patients are treated (Alberta), the type of lung cancer (NSCLC, specifically adenocarcinoma), and how many lung cancer primaries there were (one). As the human body's functions are very interconnected, there are many confounding variables that may influence the effect of EGFR. For example, the amount and type of RT[20], age[21], and other factors that alter the immune system or treatment may influence the outcome of an abscopal effect.

Method

Three hundred and thirty nine patients with non-small cell lung cancer from two cancer centres in Alberta (the Tom Baker Cancer Centre in Calgary and the Cross Cancer Institute in Edmonton) participated in a retrospective cohort study. All patients were over 18 years of age and received immunotherapy between January 1st, 2010 and June 1st, 2019. Data was collected from provincial medical records, from ARIA-MO, a management system for oncologic patients. This information on the treatment and histology of their cancer was recorded on an EXCEL spreadsheet. Dates of discovery for brain, liver, bone, and other (primarily renal, adrenal gland, and intrathoracic) metastases, white blood cell count, and date of death were also recorded and then combined with the information of the previous study.

Statistics

Of the total 339 patients studied, 100 patients were excluded as they did not have adenocarcinoma and patients with adenocarcinoma are much more likely to have an EGFR mutation.[12] A table was made to show the demographics of the whole patient cohort, comparing factors such as gender, whether they smoked or not, stage, EGFR mutation status, whether or not their cancer recurred, whether or not they received radiation, and whether or not they received systemic therapy (aka chemotherapy).

In comparing overall survival (OS), both the total time frame, between date of diagnosis and death/lost to followup, and a 5 year time frame were charted. The mean, standard error, and lower and upper bounds for the 95% confidence interval were calculated for the EGFR mutation positive (EGFR Positive) and EGFR mutation negative (EGFR Negative) groups' mean OS times using SPSS.[22] For patients' cases that were censored (or in other words for patients that were lost to follow up), their survival time was limited to the largest recorded survival time of the entire cohort.

The p-values for both chi-square and statistical significance were calculated. This process was repeated in calculating progression free survival (PFS), or how long from when treatment is started without the cancer growing or advancing, for the total time frame and a 5 year time frame.

Lastly, a table was made to compare the frequency and percentage of occurrence of certain metastases (brain, bone, liver, adrenal, intrathoracic, and other) and EGFR status. Statistical significance was calculated between the two groups. Statistical significance can be shown by method of Chi-square and statistical significance P-values. Chi-squares shows a more significant true difference between datasets when the value is further away from 0. In the case of statistical significance, it is broadly considered that values <0.05 show a true difference between datasets.

Results

Of the total 239 patients, 226 were EGFR negative while 13 were EGFR positive.

Table 1 shows the mean OS for the two EGFR groups, as well as the standard error for this calculated mean and the lower and upper bounds of the OS that would appear in 95% of the everyday population. As can be seen, the mean OS for EGFR positive patients is about 79 months (~6.5 years) lower than patients without the EGFR mutation. The standard error for these calculations are also relatively low for their respective sizes, showing the accuracy of the calculated means.

Table 1: Mean Survival Time In Months over whole time frame (EGFR + VS EGFR -)

EGFR status	Overall Survival (months)			
	Mean Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Negative	131.555	13.257	105.571	157.538
Positive	52.958	5.368	42.436	63.481
Overall	126.725	12.733	101.770	151.681

a. Estimation is limited to the largest survival time if it is censored.

Figure 1 illustrates the percent OS for both groups. The EGFR present group's OS dropped at a much faster rate than the EGFR Absent group. By the time ~70 months (~5.8 years) had passed, the EGFR present group had about a 25% lower OS than the EGFR absent group.

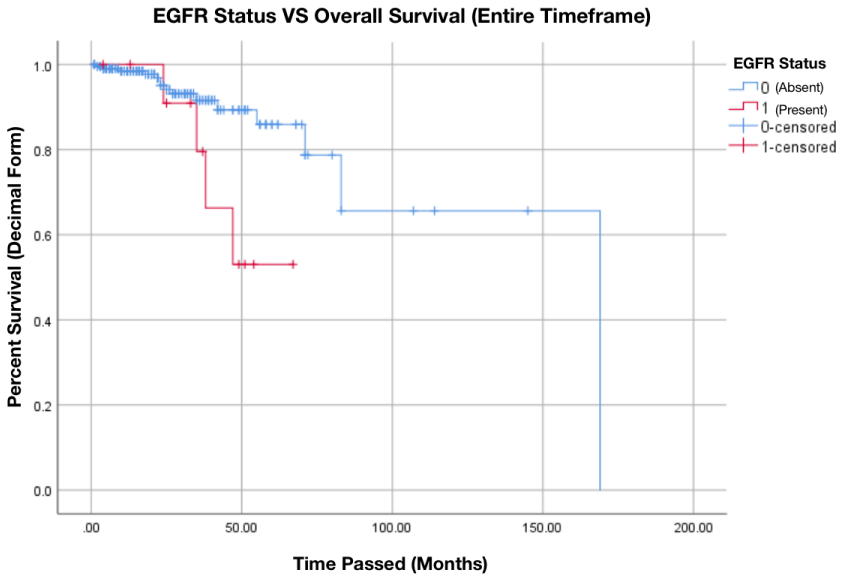

Figure 1: Overall Survival of EGFR Positive and Negative Groups Over Time (Entire Timeframe)

The blue line represents the percent overall survival of the EGFR negative group whereas the red line represents the percent overall survival of the EGFR positive group. Each small tick on the data line represents a censorship, or in other words a patient who is no longer available for follow up. After ~70 months (~5.8 years), all surviving patients had been lost to follow up and as such the data can no longer be continued to be plotted. between EGFR Positive and negative groups concerning overall survival was determined to be statistically significant (Chi-square: 5.271, p = 0.022.)

In order to see survival prognoses over smaller period of time, it is important to also look at the OS over a smaller timeframe (ie 5 years.) As can be seen in table 3, the difference between the

mean OS between the EGFR negative (56.455 months) and the EGFR positive groups (49.246 months) is now 5 months. Though this is a smaller difference than over the total time frame, it is still statistically significant (Chi-square = 5.888, p = 0.015.)

Table 3: Mean Survival Time In Months over 5 year time frame (EGFR + VS EGFR -)

EGFR Status	Overall Survival (months)			
	Mean Estimate	Std. Error	95% Confidence Interval	
			Lower	Upper
Negative	56.455	.992	54.512	58.399
Positive	49.246	4.223	40.969	57.523
Overall	55.575	1.047	53.523	57.628

a. Estimation is limited to the largest survival time if it is censored.

Figure 2 illustrates the Percent OS of both groups over the 5 year period. At 60 months, the OS of the EGFR present group is 30% lower than that of the EGFR absent group.

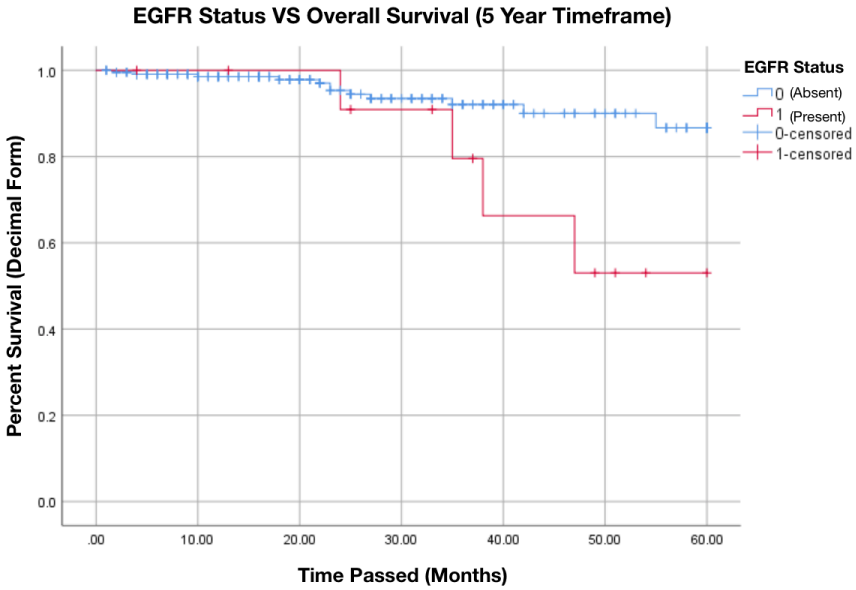

Figure 2: Overall Survival of EGFR

Table 5 shows the means of the two groups regarding PFS, or how long the patients went without the cancer growing. As can be see, there is a difference of less that 4 months between the means, with a ~2 month standard error. Although this is comparable to the difference in the 5 year OS means as seen in Table 3, it does not have statistical significance (Chi-square = 0.000, p = 0.994.) This data is illustrated in Figure 3, where the lines for the two groups are nearly identical, accounting for the more rigid changes in the EGFR present group caused by sample size.

Table 5: Mean PFS Time in Months Over Total Timeframe (EGFR + VS EGFR -)

EGFR Status	Progression Free Survival (months)			
	Mean Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Negative	18.549	2.395	13.855	23.243
Positive	14.754	2.800	9.267	20.241
Overall	18.260	2.243	13.863	22.656

a. Estimation is limited to the largest survival time if it is censored.

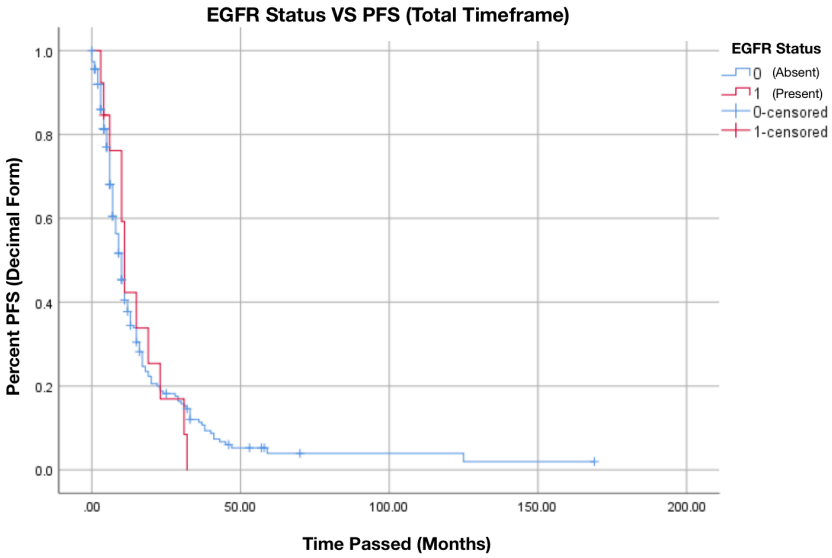

Figure 3: PFS Over Time between EGFR Positive and Negative Groups

In Table 6, EGFR status is compared with the site of a certain metastasis and whether or not it occurred. For metastasis (mets) other than brain, bone, and liver metastases, there was only a 1% difference between the two groups' frequencies of occurrence without statistical significance ($p = 0.938$.) On the other hand, the percentages of occurrence of brain and bone mets between the two groups had differences of 41.8% and 28.7% respectively. These differences were further shown to be statistically significant ($p = 0.001$ and $p = 0.044$ respectively.) Liver mets had a difference of 24.6% between the two groups, with a statistical significance trending towards being notable ($p = 0.061$.)

Table 6: Frequency of Occurrence of Different Metastases for EGFR Positive and Negative Groups

Site of Metastasis	EGFR status		
	Negative	Positive	P value
Brain			
No	164 (72.6%)	4 (30.8%)	**0.001**
Yes	62 (27.4%)	9 (69.2%)	
Liver			
No	160 (70.8%)	6 (46.2%)	0.061
Yes	66 (29.2%)	7 (53.8%)	
Bone			
No	117 (51.8%)	3 (23.1%)	**0.044**
Yes	109 (48.2%)	10 (76.9%)	
Other			
No	33 (14.6%)	2 (15.4%)	0.938
Yes	193 (85.4%)	11 (84.6%)	

Discussion

The hypothesis that patients with the EGFR mutation would have a lower likelihood of achieving an abscopal effect was supported by the results. The patients with an EGFR mutation experienced a mean OS almost one third of that of the patients without an EGFR mutation over the whole timeframe (Table 1: 52.958 vs 131.555 months). Additionally, over a 5 year timeframe, the mean OS for patients with an EGFR mutation was 5 months lower than that if the patients without

an EGFR mutation (Table 3: 56.455 vs 49.246 months). EGFR positive patients also experienced a quicker rate of change for OS (Figures 1 and 2), and a higher rate of occurrence for brain, bone, and possibly liver metastasis as compared to the EGFR negative group (Table 6.) However, there was no statistically significant difference in the PFS between the two groups.

These results regarding OS are supported by previous studies. It has been shown that EGFR-mutated patients have less of a response to immunotherapy than non-mutated patients, a trait that is unrelated to previous treatment against EGFR or with PD-L1 expression.[23] This may be due to the unique Tumour Microenvironment (TME) of EGFR-mutated tumours; there is often a lower amount of tumour-infiltrating lymphocytes[24] and a lower tumour mutational burden (TMB)[25], making the tumour cells more resemble the normal cells and so be more likely to go undetected by the immune system. It has also been shown that RT, which would normally destroy cancer cells and release TAAs, can promote tumour cell proliferation via EGFR pathways and the avoidance of apoptosis in EGFR-positive tumours, at least for a certain period of time.[26] Additionally, no significance was shown in the differences in PFS between EGFR positive and negative patients in the data of this study. This is a controversial subject, as there are conflicting studies on whether EGFR affects PFS negatively or positively, but not that it has no affect at all.[27,28] It is likely that the limits in patient cohort size for EGFR positive patients in this study caused the discrepancy in this result, and so additional testing would be required for more conclusive results.

There was also shown to be a statistically significant increase in rates of bone and bone metastases, as well as a trend towards significance with liver metastasis in patients with an EGFR mutation. It has been shown in previous studies that those with EGFR mutations are more likely to develop brain metastasis[29], which may be due to the inability of immunotherapy treatments to penetrate the blood brain barrier.[30] Although in past studies there has been evidence of bone metastases to develop in patients with an EGFR mutation from a EGFR-negative original tumour[31], there seems to be no past studies correlating EGFR-positive primaries with bone metastasis

development. Although liver metastasis did not register as statistically significant, it was trending towards significant and as such is important to explore further. It has been shown the liver metastasis is more common in EGFR-positive groups than EGFR-negative groups.[32]

A few key factors could be optimized in the future to improve the results of this study. Firstly, a more equal sample size division (in other words a larger sample for patients with the EGFR mutation) would allow the results to be more accurate. Due to the limited sample size, and that the sample was originally from a study not pertaining to the EGFR gene mutation, it was not possible to expand the number of cases available to be perfectly tailored to the study's objective. Furthermore, a study in the style of a clinical trial rather than a retrospective analysis could allow for a more in depth study of the EGFR mutation. Certain variables would be able to be controlled in a consistent manner, allowing for more accurate results. Furthermore, variables could be collected when necessary rather than having to depend on previously recorded data. This would allow for more specific, in depth analysis on factors not necessarily recorded during the average treatment of a patient.

Conclusion

In 2018, there were 18,078,957 new cases of cancer and 9,555,027 deaths caused by cancer worldwide.[33] In addition, 11.6% of these new cases and 18.4% of these deaths were lung cancer mediated. Low rates of survival[1] combined with the common delay of diagnosis due to the type of symptoms and the times at which they present[34] makes lung cancer one of the most insidious and deadly types of cancer in the world. It is key that improvements in treatment methods are developed in order to increase the comfort and life expectancy of those with lung cancer. Understanding the mechanisms of the abscopal effect and finding ways to produce it more consistently will better the

treatments of this type of cancer as well as other cancers, as this effect can occur in many types of cancer.[13]

Moreover, immunotherapy is emerging as a new addition to conventional cancer therapies, benefitting patients who previously had no available course of treatment.[35] Researchers have been studying the utilization of immune checkpoint inhibitors, combined with other treatments such as treatments against EGFR mutations, as immunotherapy options that boost the immune system rather than attack the cancer cells directly. Many developments have been made in the past 10 years or so that have spurred forth advancements in immunotherapy, but further study in how to optimize these treatments to produce a complete abscopal effect is necessary in order to utilize these new options to the fullest extent. It is also important to see how different traits of a tumour affect the treatment outcomes for patients in order to optimize treatments as best as possible.

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