Serial interferon-gamma release assay in lung cancer patients receiving immune checkpoint inhibitors: a prospective cohort study

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Running title: Serial IGRA in ICI-treated lung cancer patients

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Key point: We evaluated IGRA conversion to understand tuberculosis reactivation after ICI therapy induction in lung cancer patients. A positive IGRA status before ICI administration or IGRA conversion during ICI therapy should alert clinicians to monitor for the development of active

tuberculosis.

Abstract

Recent advancements in cancer immunotherapy using immune checkpoint inhibitors (ICIs) have received considerable attention. Although advantageous, ICI therapies cause unique immune-related adverse events (irAEs) in some patients. Moreover, infectious diseases, such as tuberculosis, have been recognized as emerging concerns during immunotherapy. We aimed to evaluate the interferon-gamma release assay (IGRA) conversion rate and active tuberculosis incidence during immunotherapy to elucidate the incidence of tuberculosis reactivation after ICI therapy induction.

We prospectively assessed IGRA results in lung cancer patients who received ICI monotherapy before ICI treatment and at 6 and 12 months after ICI treatment. We also assessed computed tomography findings to determine the presence of active tuberculosis when positive IGRA results were obtained. The ICIs used were nivolumab, pembrolizumab, atezolizumab, and durvalumab.

In all, 178 patients were prospectively recruited between March 2017 and March 2020. Of these, 123 completed serial IGRAs, of whom 18, 101, and 4, respectively, had positive, negative, and indeterminate IGRAs at baseline. Three and four patients, respectively, showed IGRA reversion and conversion during immunotherapy. One patient with a sustained, stable positive IGRA and one with

IGRA conversion developed active pulmonary tuberculosis during immunotherapy.

We found that 3.3% and 1.6% of the patients developed IGRA conversion and active tuberculosis, respectively. Of the four patients who developed IGRA conversion, one developed active pulmonary tuberculosis during immunotherapy. Another patient with sustained, stable positive IGRA developed active tuberculosis. Physicians should be alert to tuberculosis development during ICI therapy and IGRA testing is a useful tool to assess the risk of developing active tuberculosis.

INTRODUCTION

Recently, immunotherapy using immune checkpoint inhibitors (ICIs) has transformed the treatment of cancer. While these therapies are beneficial to some patients, they can cause unique immune-related adverse events (irAEs), including skin rash, hypothyroidism, pituitary dysfunction, colitis, and interstitial lung disease (1). In addition to irAEs, infections can also occur in patients receiving ICIs (2–4). The profile of infections associated with ICI therapies is unique and differs from that associated with traditional chemotherapy.

Many of the infections associated with ICIs appear to be triggered by immunosuppressive therapy for the management of irAEs (2, 4–6). However, increasing evidence suggests there might be another mechanism, independent of immunosuppression, that may predispose to infection (3, 7, 8). Previous studies have suggested that this mechanism might be the result of excessive and dysregulated host immune responses, which counterintuitively favor the pathogen (9). We have recently proposed the concept that immune dysregulation leads to some infections in patients receiving ICIs (10). Recently, there have increasing reports of tuberculosis reactivation during ICI therapy (9, 11–15). We previously reported that 1.7% of lung cancer patients who received ICI monotherapy developed active

tuberculosis in a retrospective cohort study (16).

The precise mechanisms of tuberculosis development in the context of ICI remain unclear, and the development of active tuberculosis in lung cancer patients is a major concern, as the diagnosis may be missed, leading to unfavorable outcomes, and increasing the risk of transmission of tuberculosis to other individuals. The utility of screening and monitoring for active tuberculosis before and during therapy with ICIs is not well understood. Furthermore, no prospective cohort study to date has evaluated the use of interferon-gamma release assays (IGRAs) to predict tuberculosis development in these patients, and the optimal timing of IGRA testing has not been evaluated. Hence, in this study, we aimed to evaluate the utility of serial IGRA testing during immunotherapy to understand the incidence of tuberculosis reactivation after initiation of ICI therapy and inform clinical practice.

METHODS

This prospective study was conducted at the National Hospital Organization Kyoto Medical Center, a 600-bed hospital located in Kyoto, Japan, between March 2017 and March 2021. Patients with pathologically-diagnosed lung cancer who had received ICI monotherapy for at least 12 months were

included. ICI therapy was defined as receipt of nivolumab, pembrolizumab, atezolizumab, or durvalumab. Interruption of ICI therapy was accepted in participants who developed active tuberculosis during the observation period. Prior to inclusion in the study, written informed consent was obtained from all participants. Between December 2015 and November 2018, ICI monotherapy was the only treatment option for non-small-cell lung cancer (NSCLC). After December 2018, cytotoxic chemotherapy in combination with ICIs was approved for the management of NSCLC and small cell lung cancer. To exclude the influence of cytotoxic chemotherapy, only lung cancer patients receiving ICI monotherapy were included in the analysis.

ICIs were dosed per their approved schedules: nivolumab, every 2 or 4 weeks; pembrolizumab, every 3 or 6 weeks; atezolizumab, every 3 weeks; and durvalumab, every 2 or 4 weeks. Because ICIs other than pembrolizumab were approved as second- or later-line treatments, the study participants were not restricted to the induction of cytotoxic chemotherapy before or after ICI monotherapy. Patients were assessed for IGRA status before starting ICI treatment (at baseline) and at 6 and 12 months after ICI treatment initiation. Computed tomography (CT) scans were obtained to determine the presence of active tuberculosis in patients with positive IGRA results at any time. There are two commercially available IGRAs, T-SPOT.*TB* assay (Oxford Immunotec, Oxford, UK) and QuantiFERON-TB Gold

In-Tube assay. Because only the T-SPOT.*TB* assay was approved for use at our institution, and was performed according to the manufacturer's guidelines. The result was interpreted as positive, negative, or indeterminant according to the manufacturer's guidelines.

Definitions of Serial IGRA Status

IGRA conversion was defined as a change of the IGRA result from negative to positive during follow-up testing. Reversion was defined as a change of IGRA result from positive to negative during follow-up testing. If the result of serial IGRA remained consistent during the study period, the result was defined as stable.

Ethical Approval

This study protocol was approved by the institutional review board of the National Hospital Organization Kyoto Medical Center (approval number: 16-071). The study was registered at the University Hospital Medical Information Network - Clinical Trial Registration (UMIN-CTR) center

(UMIN-CTR: 000036533). Written informed consent was obtained from all the participants. Interim analyses of this study were presented at the 2020 annual congress of the European Respiratory Society.

RESULTS

Patients' Inclusion and Characteristics

Figure 1 shows the flowchart of patient inclusion. In all, 178 patients were recruited during the study period. Of these, 20 died from disease progression prior to completion of 12 months of ICI monotherapy, 20 were switched to cytotoxic chemotherapy, eight were transferred to a palliative care unit prior to completion of the treatment course, three transferred their care to other hospitals, and four were lost to follow-up. After exclusion of these patients, 123 patients completed at least 12 months of ICI therapy and underwent IGRA testing at baseline and 6 and 12 months after initiation of ICI therapy. Study participants characteristics are presented in Table 1. The participants' mean age was 70.6 ± 8.2 years, and most were male. Adenocarcinoma (40.7%) was the most prevalent histopathology, followed by squamous cell carcinoma (32.5%), and NSCLC (not otherwise specified) (23.6%). Twelve of the

123 (9.8%) patients had driver oncogene alterations on their tumors. Pembrolizumab was the most frequently used ICI.

Serial IGRAs during Immunotherapy

Figure 2 shows the trajectory of IGRAs at each stage (A) and the results of the serial IGRAs at each stage (B). While 18 (14.6%), 4 (3.3%), and 101 (82.1%) patients, respectively, had positive, indeterminant, and negative IGRA results at baseline, the number of patients with the corresponding results at the third IGRA was 19 (15.4%), 1 (0.81%), and 103 (83.7%). Four patients (3.3%) had IGRA conversion during ICI monotherapy. One patient had IGRA conversion at 6 months, and 3 patients had conversion at 12 months. Three patients had IGRA reversion during ICI monotherapy. The remaining 116 patients maintained a stable IGRA status during ICI monotherapy.

Incidence of irAEs and systemic steroids use for irAEs

Table 2 shows the incidence of irAEs and systemic steroids use for the irAEs. The most frequent

manifestation was skin rash (34.1%), followed by interstitial pneumonia (13.0%), general fatigue (11.4%) and hypothyroidism (10.6%). Patients who developed skin rash and interstitial pneumonia were usually treated with systemic steroids.

Development of Active Tuberculosis during Immunotherapy

Table 3 shows IGRA conversion rate and development of active tuberculosis. Overall, two (1.6%) patients developed active tuberculosis during ICI monotherapy. Of the four patients with IGRA conversion, one (25%) patient developed active tuberculosis. Of the 15 patients with a stable positive IGRA, one (6.67%) patient developed active tuberculosis. No patient with IGRA reversion or with stable negative IGRA developed active tuberculosis. Of the active tuberculosis cases, one had pulmonary tuberculosis and the other mediastinal lymphadenitis. Both patients received antimycobacterial therapy after diagnosis of active tuberculosis. Of these patients, one discontinued nivolumab for 2 months after diagnosis of tuberculosis, and the other continued durvalumab until a fixed treatment cycle. Neither patient had received systemic steroids. The detailed clinical courses of these two patients are presented below.

Description of Confirmed Tuberculosis Cases

Tuberculosis case with stable positive IGRA

A 79-year-old man was diagnosed with stage 4A lung adenocarcinoma. He had a history of well-controlled diabetes mellitus. He received cytotoxic chemotherapy with carboplatin and pemetrexed as first-line therapy. After three cycles of treatment, his adenocarcinoma progressed. Four months after the initial diagnosis, the patient received nivolumab as second-line therapy. IGRA before nivolumab treatment was positive. After 5 cycles of nivolumab therapy, he developed an irAE of mild to moderate skin rash (G2), which resolved with application of steroid ointment. After 24 cycles of nivolumab therapy (6 months), IGRA remained positive. He developed a mild cough around that time. Sequential CT images showed a new reticulonodular lesion in the right lower lung lobe. To assess whether lung cancer had recurred, bronchoscopy was performed. *M. tuberculosis* was detected in the bronchial lavage fluid. The patient stopped nivolumab therapy and started anti-tuberculosis therapy. Two months after the start of anti-tuberculosis therapy, the reticulonodular lesion markedly decreased. Thus, the

patient resumed nivolumab concomitantly with anti-tuberculosis therapy and completed a 6-month course of anti-tuberculosis therapy.

Tuberculosis case with IGRA conversion

A 77-year-old woman was diagnosed with stage 3A lung cancer of an otherwise specified type. She had no history of diabetes mellitus or other immunocompromising diseases. She received concurrent chemoradiotherapy with carboplatin and paclitaxel and 60 Gy of radiotherapy. After chemoradiotherapy, she received durvalumab as consolidation immunotherapy. She did not use any immunosuppressive drugs between the onset of her lung cancer and the immunotherapy with durvalumab. She presented with mild radiation pneumonitis (G1) after 4 cycles of durvalumab but was followed without treatment. After 22 cycles of durvalumab (11 months), imaging revealed enlargement of her mediastinal lymph nodes. She had no respiratory symptoms. Histopathological examination of the tracheobronchial lymph node obtained by endobronchial ultrasound-guided transbronchial needle aspiration revealed caseating necrosis. Aspirate culture was positive for *Mycobacterium tuberculosis*. Her IGRA was negative at baseline and 6 months but became positive at 12 months after initiation of

immunotherapy with durvalumab. Hence, anti-tuberculosis therapy was started. She did not stop the durvalumab treatment as only two cycles remained until the end of the fixed durvalumab consolidation therapy. She tolerated anti-tuberculous medication and the mediastinal lymphadenopathy receded on interval scanning at 3 months.

DISCUSSION

With an increase in the use of ICIs in the treatment of advanced-stage lung cancer, reports on the development of tuberculosis have also progressively accumulated (9, 11–15). Although it is well known that opportunistic infections are among the major adverse events during treatment with immunosuppressive biologic agents such as anti-TNF agents, it is not known whether ICI therapy carries a similar risk. ESCMID guidelines suggest that immunosuppressive therapy for treating irAEs is a major risk factor for the development of infections among patients receiving ICI therapy (5, 6). In addition, several studies have suggested that ICI therapy may independently cause immune dysregulation predisposing to infection (3, 7–9). In this context, tuberculosis is one the notable infections observed in patients receiving ICI therapy (15).

Here, we performed the first prospective study in patients receiving ICI monotherapy in order to evaluate the utility of serial IGRA testing to identify patients at risk for development of active tuberculosis. Four of the 123 (3.3%) patients in the study had conversion of IGRA during ICI therapy, of whom one developed active tuberculosis. Overall, two of 123 (1.6%) patients developed active tuberculosis, a rate similar to what we had noted in a prior retrospective cohort study, where 1.7% of patients receiving ICIs developed active TB (16).

In our current study, active tuberculosis developed a patient with a stable positive IGRA and in a patient who had conversion of IGRA during ICI therapy. Both patients had experienced irAEs during immunotherapy, but neither had received systemic steroids or other immunosuppressants. In our previous study, we found that three out of five patients who developed active tuberculosis during ICI therapy had a positive IGRA status before ICI administration (16). Having a positive IGRA at baseline or conversion of IGRA during ICI therapy might be important predictors of active tuberculosis development.

Although the precise mechanisms by which ICI therapy predisposes to tuberculosis reactivation are unclear, several mechanisms have been proposed. The interaction between PD-1 and PD-L1 maintains a balance of immune tolerance (17). In cancer therapy, PD-1 inhibitors are used to disrupt this balance

of immune tolerance and induce an immune response to cancer. On the other hand, this immune tolerance may be important for conserving immune homeostasis and preventing excessive immune responses in cases of latent tuberculosis infection (18). In tuberculosis infection, the PD-1/PD-L1 interaction has historically been thought to limit an effective host immune response and contribute to the immune evasion by the pathogen (19–21). However, studies have shown that PD-1 deficient mice develop rapidly progressive tuberculosis, suggesting the harmful effect of an excessive immune response (22, 23). Supporting this, a recent report described active tuberculosis in a patient with inherited PD-1 deficiency (24). These studies and others suggest an important role for the PD-1/PD-L1 pathway in controlling tuberculosis (25–27).

The disruption of immune tolerance caused by ICI therapy may be one of the reasons for tuberculosis reactivation, with excess cytokines such as TNF-α implicated (28, 29). Elkington et al. proposed another interesting consideration (9). Clinical observations suggest an intriguing overlap between infection and autoimmune disease, with gene profiles of tuberculosis patients overlapping those from patients with autoimmune disease, implying a common underlying mechanism (30). The pathology of active tuberculosis could potentially be accentuated by an over-reactive immune response (30, 31), similar to the autoimmune phenomenon seen in patients receiving ICI therapy. The mechanisms

described above are presently hypothetical and will need to be investigated in future studies.

Although some patients in our study had IGRA reversion, the significance of reversion is unclear. IGRAs have a relatively high rate of spontaneous conversion, with a previous study in North American healthcare workers showing IGRA conversion rates of 5% to 8% (32, 33). In this study, the conversion or reversion rate may have affected the possible false-positive or negative cases. Nonetheless, the fact that a quarter of the patients showing conversion developed active tuberculosis suggests that IGRA conversion can be a marker for increased risk of tuberculosis development. Furthermore, in this study, there were 13 patients with indeterminant T-SPOT.*TB* results. Six of these patients were receiving systemic steroids for the treatment of irAEs, likely contributing to these indeterminant results (34).

In this study, we did not administer treatment for latent tuberculosis in patients with positive IGRA results. Currently, there are no agreed guidelines on the need for pre-treatment IGRA testing and chemoprophylaxis for positive results among patients receiving lung cancer treatment (4). Others have suggested that patients receiving cancer immunotherapy should undergo screening for latent tuberculosis and receive treatment if positive (4, 13). Although our study focused on lung cancer, active tuberculosis has be also been reported in patients receiving ICI therapy for other cancer (10), and it seems likely that this phenomenon is independent of the cancer type.

This study has several limitations. First, it was conducted at a single medical center in Kyoto. Thus, the results may not be applicable to other settings. Kyoto City is an area with moderate tuberculosis burden, with an annual incidence of tuberculosis in 2019 of 15.1 cases per 100,000 people (35). The majority of cases of tuberculosis occur in domestic residents aged 70 years and higher, and age group that overlaps with the peak age of lung cancer onset. The fact that 14.6% of our study participants had a positive IGRA result at baseline, reflection the epidemiology of tuberculosis in Kyoto City. Our results might therefore not be applicable to countries and regions with a lower burden of tuberculosis. Nonetheless, clinicians living in areas with a moderate to high tuberculosis incidence should be alert to the risk of development of active tuberculosis among patients receiving ICI therapy. Second, we only used the T-SPOT.*TB* assay for the diagnosis of latent tuberculosis infection. It is suggested that the combined use of both IGRA and the tuberculin skin test (TST) could increase the sensitivity of the assays among immunocompromised hosts or high-risk subjects (36, 37). Because Bacillus Calmette-Guerin (BCG) vaccination for tuberculosis is compulsory in Japan, positive TST results in the absence of latent tuberculosis infection are common. Consequently, TST is less useful for people living in countries with universal BCG vaccination, such as Japan. Third, our study was limited to an observation period of 12 months. A longer observation period might have led to the diagnosis of more

active tuberculosis cases. Thus, we may have underestimated the true incidence of tuberculosis among patients receiving ICI therapy.

In conclusion, we described prospective serial IGRA analysis in ICI-treated patients with lung cancer. A positive IGRA result before ICI administration or IGRA conversion during ICI therapy may predict development of active tuberculosis in these patients. In this new era of cancer immunotherapy, clinicians need to be aware that infections such as tuberculosis could result not only from immunosuppression associated with traditional chemotherapy but also from immune dysregulation or an over-exuberant immune response associated with ICI therapy (10). IGRA testing before and during ICI treatment may identify patients at higher risk for development of active tuberculosis and could inform initiation of prophylactic treatment.

Declarations

Ethics approval and consent to participants

This study protocol was approved by the institutional review board of the National Hospital

Organization Kyoto Medical Center (approval number: 16-071). Written informed consent was obtained from all the participants.

Availability of data and material

Data in this study can be provided with ethical committee approval if there is a rational reason.

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Conflicts of interest

All authors: No conflict.

Authors' contributions

Study concept and design: KF, PE, GRS, KN, and TM

Recruitment of subjects and data acquisition: KF, OK, YY, TI, MO, KN, and TM

Analysis and interpretation of data: KF, PE, GRS, OK, YY and TM

Preparation of drafted manuscript: KF, PE, GRS, OK, YY and TM.

Revision of manuscript: KF, PE, GRS, and TM

All authors have approved this manuscript for submission.

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Table 1. Characteristics of study participants.

Characteristic	n = 123
Age	70.6 ± 8.2
Sex: female	34 (27.6)
Smoking history	100 (81.3)
Histopathology	
Adenocarcinoma	50 (40.7)
Squamous cell carcinoma	40 (32.5)
Small-cell lung cancer	4 (3.3)
Not otherwise specified	29 (23.6)
Performance status (≥2)	6 (4.9)
Driver oncogene alterations	
EGFR	9 (7.3)
ALK	1 (0.81)
ROS-1	1 (0.81)
KRAS	1 (0.81)
Type of ICI*	
Nivolumab	20 (16.3)
Pembrolizumab	66 (53.7)
Atezolizumab	45 (36.6)
Durvalumab	20 (16.3)
PD-L1 expression	
TPS ≥ 50%	33 (26.8)
1 ≤ TPS < 50%	29 (23.6)
TPS < 1%	26 (21.1)
Unknown	35 (28.5)

Data are shown in number (%) or mean±SD

Abbreviations: EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS-1, ROS proto-oncogene 1; KRAS, Kirsten rat sarcoma; ICI, immune checkpoint inhibitor; PD-L1,

programmed cell death ligand 1; TPS, tumor proportion score.

* Patients who received multiple ICIs.

Table 2. Incidence of irAEs and systemic steroids use for the irAEs in this study cohort

	G2≥	G3≤	Total	Systemic steroids use for irAEs
Skin rash	38	4	42	5
Elevation of liver enzyme	10	1	11	2
Fatigue	13	1	14	1
Interstitial pneumonia	11	5	16	7
Diarrhoea	5	1	6	2
Fever	10	1	11	0
Central neurological disorder	0	3	3	3
Peripheral neurological disorder	5	0	5	0
Hypothyroidism	12	1	13	1
Adrenal hypofunction	2	2	4	2

Abbreviation: irAEs, immune-related adverse events.

Grade is shown according to the CTCAE version 5.0.

Table 3. IGRA conversion rate and incidence of active tuberculosis.

	Patients with IGRA conversion			Patients with IGRA reversion			Patients with stable IGRA	Total
	at 6 months	at 12 months	All	at 6 months	at 12 months	All		
Number of patients	1	3	4	2	1	3	116*	123
Development of active tuberculosis	0	1	1	0	0	0	1**	2

Abbreviation: IGRA, interferon-gamma release assay

*The breakdown is as follows: stable positive, 15; stable negative, 100; and stable indeterminant, 1.

**Patient with a stable positive IGRA status

Figure legends

Figure 1: Flowchart of study participants.

Figure 2: (A) Trajectory of IGRA status at baseline and 6 and 12 months after ICI monotherapy and (B) the number of the IGRA status at each stage.

IGRA: interferon-gamma release assay; ICI, immune checkpoint inhibitor

Figure 1

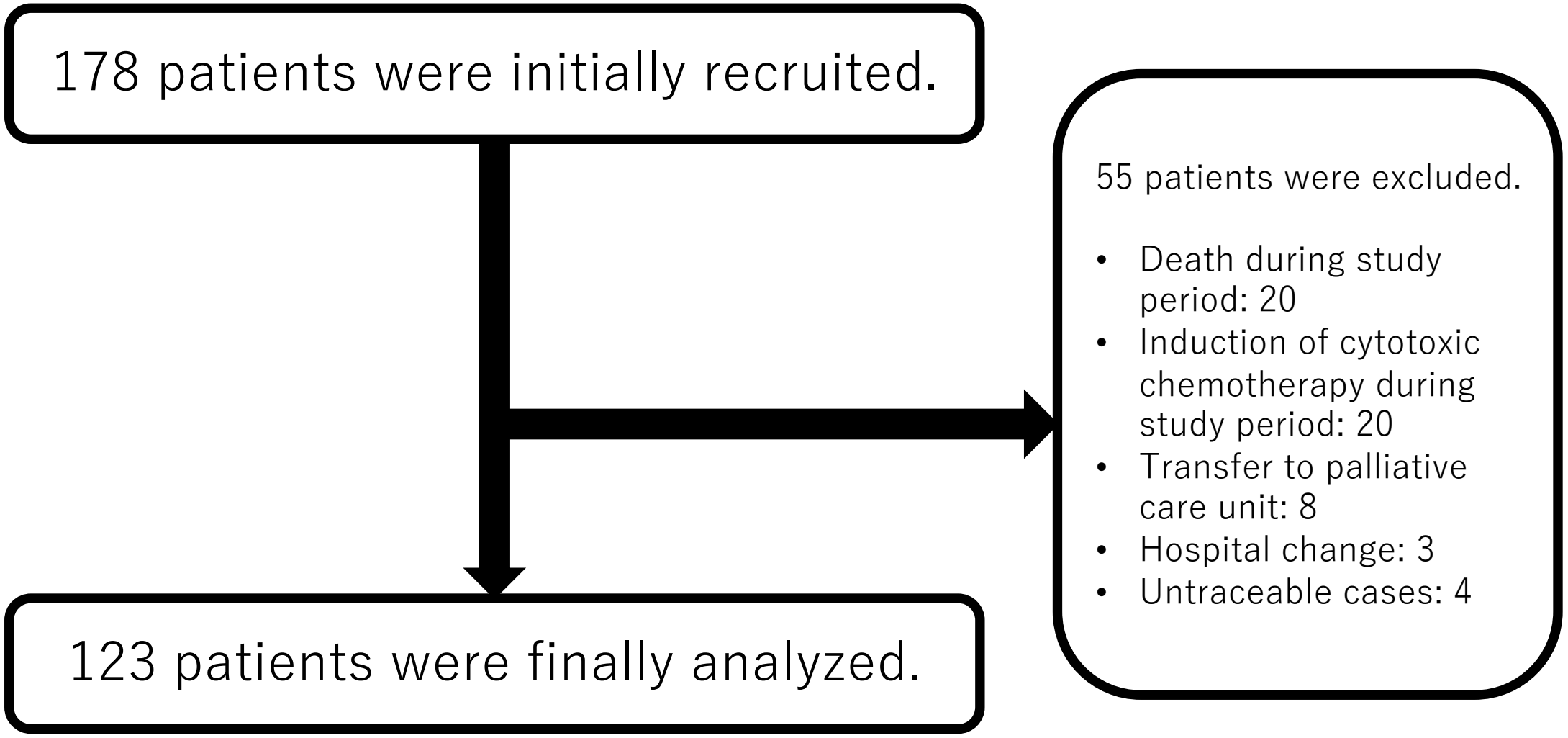

Figure 2A

Click here to access/download;Figure;Figure 2A.flowchart.pdf

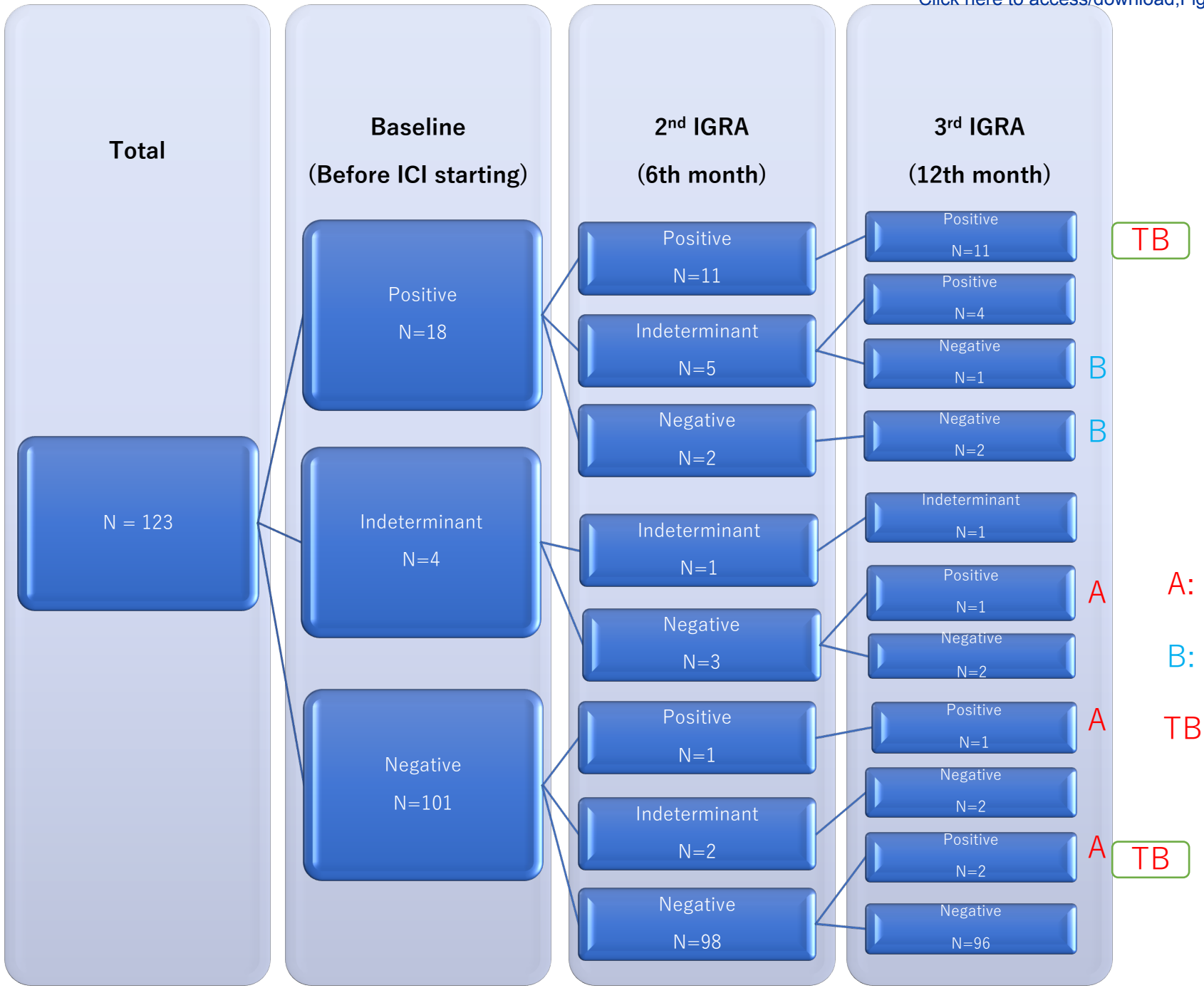

Figure 2B

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