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I have read this report and confirm that to the best of my knowledge this report accurately describes the conduct and results of the study PRJ2711: Evaluation of Rates of Suspected HSR Associated with Abacavir Using Data from the OPERA Database (eTrack Project # 206206).

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1. EXECUTIVE SUMMARY

Background: Abacavir sulfate, a carbocyclic 2'-deoxyguanosine nucleoside analog, was approved for the treatment of adults and children with HIV infection by the FDA in December 1998. Early phase trials with abacavir identified a hypersensitivity-like reaction. The exact mechanism of abacavir hypersensitivity reactions is not clearly understood, however, it is known to be a multi-organ syndrome characterized by a sign or symptom in two or more of the following categories:

Group 1: Fever
Group 2: Rash
Group 3: Gastrointestinal (nausea, vomiting, diarrhea or abdominal pain)
Group 4: Constitutional (generalized malaise, fatigue, aches)
Group 5: Respiratory (dyspnea, cough, pharyngitis)

Studies subsequently found an association between abacavir hypersensitivity and specific human leukocyte antigen (HLA) alleles. Following the identification of a genetic link, HLA-B*5701 testing entered clinical use in 2008 with the demonstration of the clinical utility of HLA screening where it was found that screening could eliminate immunologically-confirmed hypersensitivity reaction.

It has been eight years since the HLA-B*5701 test was introduced and added to guidelines for clinical care. There is need to assess the use and effectiveness of screening on the occurrence of abacavir-associated hypersensitivity reaction in real world practice.

Methods: An observational analysis of a clinical cohort utilizing prospectively-collected EMR data obtained from the OPERA Observational Database was used to address study objectives. The primary objectives of this study were:

- To describe the baseline demographic and clinical characteristics of HIV+ patients initiating an abacavir-based antiretroviral therapy (ART) regimen.
- To describe the annual incidence rates and cumulative frequencies of HLA-B*5701 testing before and after June 15, 2008.
- To describe and compare the annual incidence rates and cumulative frequencies of hypersensitivity reaction among abacavir-exposed patients before and after June 15, 2008.

OPERA patients were included who had initiated an abacavir-containing regimen for the first time between 1/1/1999 and 1/1/2016 while in the care of an OPERA caregiver. To be eligible for inclusion, patients were also required to have at least one clinic contact in the 12-months prior to abacavir initiation and at least one clinical contact in the first 12-months following abacavir initiation.

Patients included in the analysis population were observed from their index date (start date of first abacavir) until the earliest of the following events: a)

discontinuation of abacavir, b) cessation of continuous clinical activity, c) death or d) study end (07/31/2016). Patients failing to meet the continuous clinical activity requirement were censored 12 months after their last contact. Patients were compared by the HLA-B*5701 testing time period in which they initiated abacavir (Pre-HLA-B*5701 screening period: January 1, 1999 to June 14, 2008 or Post-HLA-B*5701 screening period: June 15, 2008 to July 31, 2016).

Descriptive statistics were used to detail baseline clinical and demographic characteristics. Where applicable, statistical comparisons were made using Pearson's chi-square or Fisher exact tests for categorical variables and Wilcoxon rank-sum test for continuous variables. Incidence density was described using incidence rates and compared using incidence rate ratios. Time to event was described using Kaplan Meier curves.

Each patient identified as having a diagnosis of HSR or symptoms of HSR were identified in the database. Clinical information including symptomology, potential confounders (including concurrent medications associated with these symptoms and concurrent diagnoses associated with these symptoms), as well as symptom progression were prepared anonymously for each patient and reviewed by a panel of three physicians independently. The physicians determined if the patients were a definite case, a probable case, a possible case or not a case. In the event that the physicians did not agree on case determination, the case was sent back to the physician team for discussion and determination.

Results: Of the 71,627 HIV+ patients in the OPERA database as of July 31,2016, 15,648 had initiated their first abacavir regimen between January 1, 1999 and January 1, 2016. Fewer patients (9,898; 63%) had a clinic visit with their OPERA provider in the year prior to starting their first abacavir-containing regimen. Most of these patients (97%) had at least one active visit or contact with their OPERA clinic in the 12 months following initiation of abacavir, resulting in an eligible population of **9,619** patients for analysis. One-third of the population (3,215) initiated abacavir in the pre-HLA-B*5701 period (1/1/1999 to 6/14/2008) and two-thirds (6,404) in the post-HLA-B*5701 period.

Patients in the post-HLA-B*5701 period were significantly different than those in the pre-HLA-B*5701 screening period; the differences were consistent with the changing HIV epidemic over these time periods. Patients in the pre-screening period were younger, more likely to be male, not African American or Hispanic, men who have sex with men (MSM), and from the western United States than those who initiated after screening was available. They also had higher viral loads, lower CD4 counts, were more likely to be ARV treatment-experienced, and have a history of AIDS defining illness at baseline.

The proportion of patients screened for HLA-B*5701 ever and prior to abacavir initiation has increased over time. Of patients initiating abacavir-containing regimens

in 2015 (the last full year of data), 84.3% were screened prior to an abacavir prescription compared to 40% in 2008 upon approval of the test.

Using diagnoses of HSR or symptoms of HSR, 463 (4.8%) patients were identified for review by a physician panel for HSR events (7.2% pre-screening period versus 3.5% post-screening period ($p<0.0001$)). Following adjudication by the physician panel, rates fell to 1.6% pre-screening and 0.6% post-screening ($p<0.0001$). Median time to event was 20 days (IQR: 12.0, 28.5) for both groups ($p=0.9359$).

When these events were limited to those determined to be definite or probable by the physician panel, rates were further reduced to 1.3% pre-screening and 0.4% post-screening ($p<0.0001$), and a median time to event of 17 days (IQR: 10, 27 days) which did not differ between groups ($p=0.7028$).

Incidence of HSR within 6 weeks of abacavir use decreased over time with a high in 2002 of 2.2% to a low of 0.2% in 2015 and by era with the advent of screening. The post-screening incidence density was significantly lower than (0.00016 cases per person-days; IQR: 0.00012, 0.00022) the pre-screening period of 0.00039 cases per person-days (IQR: 0.00030, 0.00052) for an incidence rate ratio of 0.36 (0.23, 0.55).

Deaths within 14 days of an adjudicated HSR event were rare occurring in 7 patients and did not differ between groups; 1 (0%) of the pre-screening population and 6 (0.1%) of the post-screening population ($p=0.4370$). However, no cause of death data was available in the diagnoses data to link these deaths to a hypersensitivity reaction.

In both sensitivity analyses, the rates of hypersensitivity reactions were increased from the main analysis. However, the same basic trends were observed. Expanding the events to include any physician adjudicated event regardless of proximity to abacavir use or evaluating any diagnosis of HSR or symptoms of HSR increased the number of events and the time to event. The latter experiencing more than 80% of events beyond 6 weeks. However, the same trend in reduced events in the post-screening period versus the pre-screening period was observed.

Conclusions: HLA*B-5701 screening has increased steadily from its introduction in 2008. Hypersensitivity reactions have decreased in the same time period. Expanded HLA*B-5701 screening in clinical practice has been associated with fewer patients experiencing abacavir-associated hypersensitivity reactions in the OPERA cohort.

2. BACKGROUND AND STUDY RATIONALE

Abacavir sulfate, a carbocyclic 2'-deoxyguanosine nucleoside analog, was approved by the FDA in December 1998, for the treatment of adults and children with HIV infection. The approval of abacavir was based on studies that showed improved CD4 profile and decreased plasma HIV RNA levels in patients who took abacavir in combination with other nucleoside analogues versus those who took antiretroviral regimens without abacavir.[1,2] Abacavir is converted intracellularly by enzymes, into the active compound carbovir triphosphate. This, in turn, competitively inhibits HIV reverse transcriptase and terminates proviral DNA chain extension.[3]

Originally marketed as Ziagen®, abacavir has since been co-formulated with two other nucleoside reverse transcriptase inhibitors, zidovudine and lamivudine (3TC), approved as Trizivir®, followed by co-formulations with lamivudine, approved as Epzicom® and with lamivudine and dolutegravir (DTG), approved as Triumeq®. With all formulations, abacavir is widely used to achieve viral suppression and immunologic improvement in patients with HIV infection. Factors that make abacavir a suitable choice for HIV therapy are its high oral bioavailability (geometric mean of absolute bioavailability is 83%), no significant effect of food on the extent of absorption, pharmacokinetics that support once daily dosing, good central nervous system penetration, no significant drug interactions, and slow development of drug-resistant mutants.[4-8]

Early phase I/II trials with abacavir indicated the occurrence of side effects like headache, gastrointestinal disturbances, rash, malaise, fatigue and asthenia. Like many antiretroviral drugs, abacavir is metabolized by cytochrome P450 in the liver. Therefore, as with other nucleoside analogues, patients who take abacavir are susceptible to lactic acidosis, hepatomegaly and steatosis.[9] Among nucleoside analogues, abacavir is believed to have a lower propensity for causing mitochondrial toxicity. Studies show that switching patients with symptomatic hyperlactatemia or lactic acidosis from stavudine and/or didanosine to abacavir and lamivudine result in less potent levels of hyperlactatemia.[10]

Hypersensitivity is the term used for an extreme form of adaptive immune response. Such responses occur when the immune system reacts inappropriately to certain antigens, and may lead to inflammatory reactions and tissue damage.[11] There are four types of hypersensitivity reactions – I, II, III and IV. Type I hypersensitivity is mediated by immunoglobulin E (IgE), leading to the release of pharmacological mediators which produce an acute inflammatory reaction. Type II hypersensitivity is antibody-dependent (IgG or IgM) and occurs when antibodies bind to self or foreign antigens on cells, causing phagocytosis, killer cell activity or complement-mediated lysis. Type III hypersensitivity develops when large immune complexes cannot be cleared from the reticuloendothelial system. Type IV or delayed type hypersensitivity (DTH) occurs when antigens are trapped in a macrophage and cannot be cleared. As a result, cytokines are released and these mediate a range of inflammatory responses.

The exact mechanism of abacavir hypersensitivity reactions is not clearly understood, although studies suggest the involvement of T-cells and the cytokines interferon-gamma (IFN-γ) and interleukin-4 (IL-4).[12,13] Hypersensitivity to abacavir is a multi-organ syndrome characterized by a sign or symptom in two or more of the following categories:

Group 1: Fever
Group 2: Rash
Group 3: Gastrointestinal (nausea, vomiting, diarrhea or abdominal pain)
Group 4: Constitutional (generalized malaise, fatigue, aches)
Group 5: Respiratory (dyspnea, cough, pharyngitis)

In a review of 9 clinical trials conducted between November 1999 and January 2002 involving 2,670 patients, 8% (range 2-9%) of patients prescribed abacavir reported a hypersensitivity reaction. The median time to onset was 9 days, with 89% presenting symptoms within the first 6 weeks of starting the drug. The vast majority of patients (95%) presented symptoms from two or more of the groups that are described above.[26]

Other symptoms of hypersensitivity include lethargy, myolysis, edema, abnormal chest X-ray, paresthesia, liver failure, renal failure, hypotension, adult respiratory distress syndrome, respiratory failure and death. Reports of anaphylaxis with initial and re-challenge exposure to abacavir have been documented.[14-17] There have also been isolated case reports of unusual symptoms like anorexia, peri-tonsillar abscess, agranulocytosis, lip ulcers and neuropsychiatric symptoms like night sweats, depression, and auditory hallucinations.[18-20] A retrospective review of data from 200,000 patients who received abacavir through clinical trials or by prescription initially identified a total of 1,803 cases of hypersensitivity to the drug. Upon further review of these cases, the calculated incidence rate in the clinical trials was determined to be 4.3%. The mortality rate in patients who received abacavir in clinical trials was 0.03%.[21]

Barring rare hypersensitivity reactions with fatal outcomes, in general, the symptoms are reversed after the discontinuation of abacavir. However, hypersensitivity reaction is much more severe and more frequently lethal in patients who, after the resolution of initial symptoms, are reintroduced to abacavir. Following a diagnosis of hypersensitivity, patients must not take abacavir again. Restarting the drug following a hypersensitivity reaction has resulted in cases of life-threatening hypotension and fatal reactions. Additionally, there have been reports of individuals who developed re-challenge hypersensitivity to abacavir after having been asymptomatic following initial use of the drug as well.[15,22] Therefore, it is recommended that all patients receiving abacavir be monitored closely for signs of a hypersensitivity reaction, especially in the initial weeks of treatment.[23]

Early studies examining the demographic and clinical predictors of hypersensitivity found higher risks for white race, female gender, elevated baseline CD8 and lower risks for antiretroviral treatment and African American descent.[24-27] Genetic

susceptibility factors have been suggested because of the occurrence of the reaction in a small sub-population of patients receiving abacavir, familial disposition, the low incidence of the reaction in patients of African American origin and involvement of the major histocompatibility complex (MHC) alleles in other similar multi-organ hypersensitivity reactions.[28,29] Later studies have found an association between abacavir hypersensitivity and specific human leukocyte antigen (HLA) alleles.[30]

Following the identification of a genetic link to abacavir hypersensitivity reaction, HLA-B*5701 testing entered clinical use in 2008 with the demonstration of the clinical utility of HLA screening where it was found that screening eliminated immunologically confirmed hypersensitivity reaction with a negative predictive value of 100% and a positive predictive value of 47.9%.[30] Guidelines subsequently recommended HLA testing for all patients when considering an abacavir-containing regimen.

It has been eight years since the HLA-B*5701 test was introduced and added to guidelines for clinical care. There was need to assess the use and effectiveness of screening on the occurrence of abacavir hypersensitivity reaction in a real world setting.

3. STUDY OBJECTIVES

3.1. Primary Objectives

1. To describe the baseline demographic and clinical characteristics of HIV+ patients initiating an abacavir-based antiretroviral therapy (ART) regimen.

2. To describe the annual incidence rates and cumulative frequencies of HLA-B*5701 testing before and after June 15, 2008.

3. To describe and compare the annual incidence rates and cumulative frequencies of hypersensitivity reaction among abacavir-exposed patients before and after June 15, 2008.

4. METHODS

4.1. Study Population

The study sample was identified from the OPERA Observational Database for analysis according to the inclusion criteria defined below.

Patients initiating an abacavir-containing regimen for the first time between January 1, 1999 and January 1, 2016 were included in the study sample if they met the following inclusion criteria:

1) A diagnosis of HIV-1, a positive HIV-1 Western Blot, or a positive HIV-1 enzyme-linked immunosorbent assay (ELISA); and a detectable HIV-1 viral load test.

2) At least 13 years of age at the index date.

3) Continuous clinical activity in the year prior to abacavir initiation, defined as at least one clinic visit.

4) Continuous clinical activity in the year following abacavir initiation, defined as at least one clinical contact (visit or telephone contact)

4.2. Study Design

An observational analysis of a clinical cohort utilizing prospectively-collected EMR data obtained from the OPERA Observational Database was used to address study objectives. Study participants were identified from the most recent database build available. The observation period began on January 1, 1999 (the date Ziagen® (abacavir) became widely available) and proceeded until the data were frozen for aggregation into the database (July 31, 2016). Study participants were included into the analysis population through January 1, 2016 to allow a minimum of 6 months of potential follow up for all those followed. Patients were compared by the HLA-B*5701 testing time period in which they initiated abacavir.

Comparison Time Periods:

Pre-HLA-B*5701 screening period: January 1, 1999 to June 14, 2008

Post-HLA-B*5701 screening period: June 15, 2008 to July 31, 2016

Index date: The index date for an eligible patient was defined as the first date of the first abacavir-containing regimen ever prescribed to a patient.

Baseline period: The 12-month baseline period preceding the index date was used to assess patient demographic and clinical characteristics.

Observation period: Patients were observed from their index date until the first of the following censoring events: a) discontinuation of abacavir, b) cessation of continuous clinical activity, c) death or d) study end (July 31, 2016). Patients failing to meet the continuous clinical activity requirement were censored 12 months after their last contact.

4.3. Exposure Definitions

Eligible patients who have initiated treatment with an abacavir-containing regimen after their first active visit in the OPERA database were identified. The index abacavir regimen was defined as the first abacavir regimen a patient received.

All FDA approved ARTs and formulations are present in the OPERA Observational Database from the time they are approved by the FDA. Any regimen with abacavir was included in the analysis and described. HIV+ individuals taking abacavir in any formulation (as Ziagen®, Epzicom®, Trizivir®, or Triumeq®) and in combination with any other antiretroviral drugs were eligible for inclusion.

The duration of the regimen was defined by discontinuation of abacavir. Changes to other medications within the ART regimen were not considered regimen changes as long as the patient remained on abacavir.

4.4. Study Endpoints

1. Physician-adjudicated hypersensitivity reaction defined as:
    - A diagnosis of abacavir-associated hypersensitivity reaction by the care provider within 6 weeks of abacavir initiation OR
    - A constellation of symptoms consistent with abacavir-associated hypersensitivity reaction including; abdominal pain, allergic reaction, cough, drug reaction, diarrhea, drug reaction, dyspnea, fatigue, flushing, headache, hypersensitivity, malaise, nausea, pharyngitis, rash, or vomiting within 6 weeks of abacavir initiation OR
    - A death within 14 days of a diagnosis or symptom of hypersensitivity reaction (cause of death data was not available)

2. Documentation of HLA-B*5701 testing

3. Abacavir exposure after an HLA-B*5701 positive test result

4.5. Statistical Analysis

4.5.1. Analysis of demographics/baseline characteristics

Data describing clinical and demographic patient characteristics are presented using descriptive statistics. Results are summarized using medians with interquartile ranges (IQR) for continuous variables and as frequencies and proportions for categorical variables. Where applicable, statistical comparisons of patient characteristics by HLA-B*5701 testing time period are made using Pearson's chi-square or Fisher exact tests for categorical variables and Wilcoxon rank-sum test for continuous variables.

4.5.2. Analysis of primary objectives

Results are summarized using frequencies and proportions for continuous outcomes and medians with IQR for continuous outcomes. Comparisons of the number and proportion of patients experiencing HLA-B*5701 testing and abacavir HSR events are made using Pearson's chi-square or Fisher exact tests.

Kaplan Meier methods were used to assess time to abacavir-associated hypersensitivity reaction prior to HLA-B*5701 testing availability and after testing was available.

Incidence density was described using incidence rates by year and compared over testing periods using incidence rate ratios with 95% confidence intervals.

4.5.3. Sensitivity analyses of primary objectives

The literature on abacavir-associated hypersensitivity suggests that most (90%) events will occur in the first 6 weeks after initiation of abacavir[21]. Therefore, late events are more likely to be false-positive events. To evaluate the impact of these events on the findings, a sensitivity analysis was undertaken including all events regardless of their proximity to abacavir initiation. A comparison of frequency and proportion of HLA-B*5701 testing and abacavir-associated hypersensitivity reactions were evaluated using physician adjudicated HSR events from any time after abacavir initiation.

Over time, physicians have become much more adept at identifying hypersensitivity reactions. To assess the impact of the differential skill of our physician panel at identifying events in 2016, an additional sensitivity analysis was executed including all diagnoses of hypersensitivity reaction and all symptoms consistent with hypersensitivity reaction without regard to the physician panels judgement to observe the rate and distribution of events as recorded by the treating physician with the information they had before them.

5. RESULTS

5.1. Study Population

- Of the 71,627 HIV+ patients in the OPERA Database at the data freeze date (July 31, 2016), 15,648 had initiated their first abacavir regimen between January 1, 1999 and January 1, 2016.
- Fewer patients (9,898; 63%) had a clinic visit in the year prior to starting their first abacavir-containing regimen.
- Most of these patients (97%) had at least one active visit or contact with their OPERA clinic in the 12 months following initiation of abacavir, resulting in an eligible population of **9,619** patients for analysis. (Table 1a)
- One third of the population (3,215) initiated abacavir in the pre-HLA-B*5701 period (1/1/1999 to 6/14/2008) and two-thirds (6,404) in the post-HLA-B*5701 period. (Table 1b)

5.2. Baseline Demographics Characteristics

- Patients in the post-HLA-B*5701 period were significantly different in their baseline demographic characteristics than those in the pre-HLA-B*5701 period. (Table 2)
- The differences observed between the two time periods were consistent with the changing HIV epidemic.

- **Age:**
  - Median age at baseline for all abacavir initators was 43 years (IQR: 35,50). Abacavir initiators in the pre-HLA-B*5701 period were younger [40 years (35,46)] than abacavir initiators in the post-HLA-B*5701 period [45 years (35, 52) p<0.0001].
  - Twice as many post-HLA-B*5701 initiators were 50 or older compared to pre-HLA-B*5701 initiators (31% vs. 16%, p<0.0001).

- **Sex:**
  - A larger proportion of the pre-screening abacavir initiators were male (86% vs. 83%, p<0.0001) but both groups were predominantly made up of men.

- **Race**:
  - The majority of abacavir initiators were not African American.
  - Patients initiating after HLA testing was available were more likely to be African American (37%) than those initiating prior to screening (27%, p<0.0001).

- **Ethnicity:**
  - Similarly, a minority of patients initiating abacavir self-identified as being of Hispanic ethnicity.
  - Nearly a quarter of initiators (24%) in the post-screening period were of Hispanic ethnicity as compared to the pre-screening period (19%, p<0.0001).

- **Risk of Infection:**
  - Patients in the pre-HLA-B*5701 screening period were more likely to be men who have sex with men (MSM) than in the post-HLA-B*5701 screening period (61% vs. 47%, p<0.0001).

- **Region of Care:**
  - Patients initiating abacavir before HLA testing was available were more likely to be from the western United States (70% vs. 40%, p<0.0001).
  - Patients initiating abacavir after HLA testing was available were more likely to be even split between the southern states and the western states (53% and 40%, respectively).

- **Marital Status:**
  - Most patients were single (67%) or married/in domestic partnership (12%) at initiation of abacavir.
  - Post HLA screening patients were more likely to be single (68%) or separated/divorced (3%) than post-screening patients (single, 64% and separated/divorced,2%, p<0.0001).
  - However, no conclusions can be drawn about marital status because a significant proportion of this population was missing marital status information (19%) with fewer patients in the pre-HLA-B*5701 testing period having this information available (22% vs. 18%).

- **Payer:**
  - Payer type was not mutually exclusive. In fact, many patients had more than one payer.
  - The proportion of patients on Medicaid was not significantly different for patients receiving abacavir for the first time in pre- versus the post-screening periods.
  - Slightly more patients in the pre-screening period were using Medicare than in the post-screening period (14% vs.12%, p=0.0027).
  - Commercial insurance coverage increased in the post-HLA-B*5701 period compared to the pre-HLA-B*5701 period (35% vs. 16%, p<.0001).

- A larger proportion of abacavir initiators paid some or all of their healthcare costs with cash regardless of the time period (pre-screening 42% vs. post-screening 60%, p<0.0001).
- ADAP/Ryan White coverage was more common in abacavir initiators in the post HLA-B*5701 screening period compared to the pre-screeing period (29% vs 17%, p<0.0001).
- However, many patients in the pre-screening period did not have payer information as electronic practice management systems became more widespread recently (42%).

5.3. Baseline Clinical Characteristics

- Differences between the pre-screening and post-screening patients persisted in the baseline clinical characteristics. (Table 3)
- Patients initiating abacavir during the post-HLA-B*5701 screening period were more likely to be naïve to ARV therapy prior to abacavir than patients in the pre-HLA-B*5701 screening period (43% vs. 37%, p<0.0001).
  - Among treatment-experienced initiators, pre-HLA-B*5701 patients had a greater number of regimens than post-HLA-B*5701 patients [median 2 regimens (IQR: 1,5) vs. 1 (1,3) p<0.0001].
  - Among treatment-experienced inititators, there was more time between first ART and start of abacavir for pre-HLA-B*5701 period patients [26.9 months (9.0, 66.7) vs. 21.8 (6.7, 55.3), p<0.0001] also.
- Baseline viral loads were much higher for patients initiating abacavir in the pre-HLA-B*5701 period as one would expect as viral test sensitivity differed back then and fewer therapeutic options meant that low level viremia may have been tolerated for longer.
  - Median viral load at start of abacavir was 8,051 copies/mL (151, 71,070) for pre-HLA-B*5701 screening period patients and 110 copies/mL (19, 29,462) for patients in the post-screening period (p<0.0001).
  - Nearly half of post-screening period patients had an undetectable or very low viral load (<200 copies/mL) when they initiated abacavir (48%) compared to the pre-screening period patients (23%). Moderate viral loads (>=10,000 to <100,000 copies /mL) were more common at baseline in the pre-screening patients (32%) versus the post-screening patients (19%), p<0.0001.
  - Twice as many patients were lacking a baseline viral load in the pre-screening patients than in the post-screening patients (15% vs. 8%, p<0.0001).
- Baseline CD4 lymphocyte counts were similarly disparate between the groups due to differing treatment guidelines over the years. Patients initiating

abacavir in the pre-HLA-B*5701 screening period had lower baseline CD4 counts.
    - Median CD4 count at start of abacavir were lower [274 cells/mm^3 (142, 452)] for pre-screening period patients compared to the post-screening period patients [452 cells/mm^3 (270,660), p<0.0001].
    - Many post-screening period patients had baseline CD4 counts >500 cells/mm^3 (41%) compared to many fewer in the pre-screening period patient population (18%). Conversely, many more pre-screening patients had a baseline CD4 counts below 200 cells/mm^3 (31%) compared to post-screening patients (17%, p<0.0001).
- Twice as many pre-screening patients initiated abacavir with a history of an AIDS defining illness than patients in the post-screening period (30% vs. 15%, p<0.0001).

5.4. HLA-B*5701 testing characteristics

- As one would expect, patients prescribed abacavir before the HLA-B*5701 screening test was available were far less likely to ever be screened than those who were prescribed after the test became available (23.9% vs. 72.5%, p<0.0001). (Table 4a)
    - The majority (4,343, 93.5%) of the post-screening period patients were tested before abacavir initiation. The median time between testing and initiation of abacavir was 44 days but with a huge variability (16, 370) suggesting that some caregivers are testing early so they have abacavir as an option in the future.
    - Twenty-seven patients tested positive for HLA-B*5701 and still went on to start an abacavir-containing regimen. Two of the 27 went on to develop a hypersensitivity reaction; one at day 16 and one at day 203 of abacavir use.
    - A minority (187, 4.0%) of post-screening period patients were tested after initiating abacavir after a median 104 days (28, 536) of which 3 patients were found to be positive for HLA-B*5701.
    - A few (115, 2.5%) post-screening period patients were tested after discontinuing abacavir for the first time of which 4 patients were found to be positive for HLA-B*5701.
- The proportion of patients screened for HLA-B*5701 ever and prior to abacavir initiation has steadily increased over time. (Figures 1 & 2) Of patients initiating abacavir-containing regimens in 2015, 85.9% were ever tested and 84.3% were tested prior to an abacavir prescription. (Table 4b)

5.5. Main Analysis: Hypersensitivity reaction events within 6 weeks of abacavir initiation adjudicated by a physician panel

- Using diagnoses of HSR or symptoms of HSR, 463 (4.8%) patients were identified for review by a physician panel for HSR events (7.2% pre-screening period versus 3.5% post-screening period (p<0.0001)). Following adjudication, rates fell to 1.6% pre-screening and 0.6% post-screening (p<0.0001). Eighty-eight (0.9%) patients were determined to have possibly had an HSR by the physician panel. Median time to event was 20 days (IQR: 12.0, 28.5) for both groups (p=0.9359). (Table 5a)
    - When these events were limited to those determined to be definite or probable by the physician panel, rates were further reduced to 1.3% pre-screening and 0.4% post-screening (p<0.0001), and a median time to event of 17 days (IQR: 10, 27 days) which did not differ between groups (p=0.7028).
    - Deaths within 14 days of an adjudicated HSR event were rare occurring in 7 patients and did not differ between groups; 1 (0%) of the pre-screening population and 6 (0.1%) of the post-screening population (p=0.4370). No cause of death data was available in the diagnoses data to link these deaths to a hypersensitivity reaction though.
- Incidence of HSR within 6 weeks of abacavir use by year decreased over time with a high in 2002 of 2.2% to a low of 0.2% in 2015 (Table 5b) and by era with the advent of screening in 2008 the rate continued to decline from 0.8% to 0.2% in 2015 (Figure 3).
- The post-screening incidence density was significantly lower than (0.00016 cases per person-days; IQR: 0.00012, 0.00022) the pre-screening period of 0.00039 cases per person-days (IQR: 0.00030, 0.00052) for an incidence rate ratio of 0.36 (0.23, 0.55). (Table 5c)
- Of patients experiencing an HSR event, a significant proportion were screened (34; 38.6%) mostly after exposure to abacavir. Few (4; 12%) of those screened tested positive suggesting that these rates are likely inflated versus the true rate of HSR. (Table 5d)
- Kaplan-Meier curves depicting time to physician adjudicated definite or probable HSR event were restricted to 0.90 to 1.00 probability of remaining HSR-free to be able to see the difference in the curves. Pre-screening events occurred significantly sooner than post-screening events (logrank p<.0001) but were rare events in both eras. (Figure 4)

5.6. Sensitivity Analysis: Hypersensitivity reaction events adjudicated by physician panel regardless of timing

- Reviewing all potential hypersensitivity reactions or symptoms consistent with hypersensitivity reaction without regard to the timing since abacavir initiation, the physician panel identified 191 (2.0%) patients with a definite, probable, or possible HSR event; more than doubling the number of events in both eras. As expected, the time to event also lengthened. (Table 6a-d, Figure 4)
    - Among patients initiating abacavir in the pre-HLA-B*5701 period, an event rate of 2.8% was observed with a median 35 days (17, 82) from start to event.
    - Among patients initiating abacavir in the post-HLA-B*5701 period, an event rate of 1.6% was observed with a median 69 days (27, 154) from start to event.
    - Events continued to trend down over time.
    - The post-screening period had a significantly lower event rate ($p<0.0001$) and the median time to event was shorter in the pre-screening period than the post ($p=0.004$). Only in the post-screening period events were the majority of events more than 6 weeks after abacavir initiation (63%).
- When restricting to the definite or probable events, both groups had fewer events; pre-HLA-B*5701 testing patients experienced 53 events compared to only 34 event in the post-HLA-B5701 testing patients ($p=0.0005$).
    - Days to event shrunk to a median of 23 days (13, 36) and did not differ by group.
    - Fewer events (19) occurred greater than 6 weeks after abacavir initiation and did not differ by group.
    - Possible events made up about half of events and over 75% of the events greater than 6 weeks after abacavir initiation in both groups.
- Death within 14 days of an event remained rare in this sensitivity analysis with 3 in the pre-screening group and 13 in the post-screening group ($p=0.0193$).
- Incidence densities for hypersensitivity reactions could not be calculated for this analysis because the long time to event made the densities infinitesimally small and impossible to compare.

5.7. Sensitivity Analysis: Hypersensitivity reaction diagnoses and symptoms of hypersensitivity reaction diagnoses

- Using diagnoses of hypersensitivity reaction or symptoms consistent with hypersensitivity reaction, 463 (4.8%) patients who initiated abacavir for the first time were identified to have an HSR event. (Tables 7a-d, Figure 5)

  - Among patients initiating abacavir in the pre-HLA-B*5701 period, an event rate of 7.3% was observed with a median 242 days (44.5, 648) from start to event.
  - Among patients initiating abacavir in the post-HLA-B*5701 period, an event rate of 3.5% was observed with a median 250 days (83, 579) from start to event.
  - Event rates tended to trend down over time with increased testing but were more sporadic.
  - The post-screening period had a significantly lower event rate ($p<0.0001$) but the median time to event did not differ from the pre-screening period.
  - A majority of events were more than 6 weeks after abacavir initiation in both groups.
- When restricting to definite or probable events, both groups had far fewer events; pre-HLA-B*5701 testing patients experienced 18 events compared to only 1 event in the post-HLA-B5701 testing patients ($p<0.0001$).
  - Days to event shrunk to a median of 18 days (11, 30).
  - Far fewer events (4) occurred greater than 6 weeks after abacavir initiation.
  - Possible events made up the vast majority of events and over 80% of the events greater than 6 weeks after abacavir initiation in both groups.
- Death within 14 days of an event was experienced by 3 patient in the pre-screening period and 3 patients in the post-screening period (1.000).
- Incidence densities for hypersensitivity reactions were very small due to the unexpected number of events more than 6 weeks after abacavir initation. Patients in the post-HLA*B-5701 screening period were marginally lower incidence density (IRR=0.83, IQR: 0.69, 1.00) compared to the pre-HLA*5701 period patients. Definite and probable event densities could not be calculated.

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8. TABLES AND FIGURES

8.1. Study Population

8.1.1. Table 1a: Identification of the study population

		Patients Included	%	Patients Excluded	%
1	Patients who are HIV+	71,627	.	0	.
2	Patients with HIV-1 infection (excluding HIV-2 infection)	71,556	99.9	71	0.1
3	Patients with HIV meds	63,028	88.1	8,528	11.9
4	Patients who started first regimen with abacavir between 1/1/1999 and 1/1/2016, inclusive	15,648	24.8	47,380	75.2
5	Patients who were 13 years of age or older at first abacavir-containing regimen	15,645	100.0	3	0.0
6	Patients with at least one active visit within 12 months prior to start of first abacavir-containing regimen	9,898	63.3	5,747	36.7
7	Patients with at least one active contact within 12 months after start of first abacavir-containing regimen or who died in first 12 months	9,619	97.2	279	2.8

8.1.2. Table 1b: Study population by HLA-B*5701 screening period

	N	%
Pre-HLA-B*5701 screening period (1/1/1999 – 06/14/2008)	3215	33.4
Post-HLA-B*5701 screening period (06/15/2008- 1/1/2016)	6404	66.6
Total	9619	100.0

8.2. Table 2: Baseline demographics of patients initiating treatment with abacavir

	All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Age				
Median (IQR)	42.8 (34.7, 50.5)	40.4 (34.9, 46.4)	44.6 (34.6, 52.0)	<.0001
13-25	670 (7.0%)	127 (4.0%)	543 (8.5%)	<.0001
26-49	6424 (66.8%)	2577 (80.2%)	3847 (60.1%)	
50+	2525 (26.3%)	511 (15.9%)	2014 (31.4%)	
Sex				
Male	8089 (84.4%)	2759 (86.5%)	5330 (83.3%)	<.0001
Female	1500 (15.6%)	429 (13.5%)	1071 (16.7%)	
Race				
African American	3240 (33.7%)	865 (26.9%)	2375 (37.1%)	<.0001
Not African American	6379 (66.3%)	2350 (73.1%)	4029 (62.9%)	.
Ethnicity				
Hispanic	2161 (22.5%)	615 (19.1%)	1546 (24.1%)	<.0001
Not Hispanic	7458 (77.5%)	2600 (80.9%)	4858 (75.9%)	.
Marital Status				
Single	6413 (66.7%)	2069 (64.4%)	4344 (67.8%)	<.0001
Married	652 (6.8%)	233 (7.2%)	419 (6.5%)	
Domestic partnership	332 (3.5%)	108 (3.4%)	224 (3.5%)	
Widowed	94 (1.0%)	27 (0.8%)	67 (1.0%)	
Separated/divorced	266 (2.8%)	59 (1.8%)	207 (3.2%)	
Unknown	1862 (19.4%)	719 (22.4%)	1143 (17.8%)	

	All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Risk of Infection				
MSM	5014 (52.1%)	1969 (61.2%)	3045 (47.5%)	<.0001
Not MSM	4605 (47.9%)	1246 (38.8%)	3359 (52.5%)	.
Region				
Northeast	397 (4.1%)	40 (1.2%)	357 (5.6%)	<.0001
South	4330 (45.0%)	917 (28.5%)	3413 (53.3%)	
Midwest	52 (0.5%)	0 (0%)	52 (0.8%)	
West	4840 (50.3%)	2258 (70.2%)	2582 (40.3%)	
Medicaid				
Yes	2108 (21.9%)	712 (22.1%)	1396 (21.8%)	0.6977
Medicare				
Yes	1220 (12.7%)	454 (14.1%)	766 (12.0%)	0.0027
Commercial Insurance				
Yes	2745 (28.5%)	504 (15.7%)	2241 (35.0%)	<.0001
Cash				
Yes	5168 (53.7%)	1343 (41.8%)	3825 (59.7%)	<.0001
ADAP/Ryan White				
Yes	2427 (25.2%)	546 (17.0%)	1881 (29.4%)	<.0001
Other				
Yes	57 (0.6%)	36 (1.1%)	21 (0.3%)	<.0001
No Payer info				
Yes	2495 (25.9%)	1360 (42.3%)	1135 (17.7%)	<.0001

8.3. Table 3: Baseline clinical characteristics of patients initiating treatment with abacavir

	All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Treatment naïve prior to ABC initiation				
Yes	3940 (41.0%)	1188 (37.0%)	2752 (43.0%)	<.0001
No	5679 (59.0%)	2027 (63.0%)	3652 (57.0%)	
Among experienced patients, number of regimens prior to ABC regimen				
Median (IQR)	2.0 (1.0, 4.0)	2.0 (1.0, 5.0)	1.0 (1.0, 3.0)	<.0001
Among experienced patients, time between ART initiation and ABC initiation (months)				
Median (IQR)	23.3 (7.5, 58.6)	26.9 (9.0, 66.7)	21.8 (6.7, 55.3)	<.0001
Baseline Viral Load				
Median (IQR)	685.0 (47.0, 43755.0)	8051.5 (151.0, 71079.5)	110.0 (19.0, 29462.0)	<.0001
Undetectable (<50 copies/mL)	2980 (31.0%)	355 (11.0%)	2625 (41.0%)	<.0001
Very Low (>=50 and <=200 copies/mL)	846 (8.8%)	372 (11.6%)	474 (7.4%)	
Low (>200 and <10,000 copies/mL)	1496 (15.6%)	671 (20.9%)	825 (12.9%)	
Moderate (>=10,000 to <100,000 copies/mL)	2236 (23.2%)	1024 (31.9%)	1212 (18.9%)	
High (>=100,000 copies/mL)	1043 (10.8%)	306 (9.5%)	737 (11.5%)	
Missing	1018 (10.6%)	487 (15.1%)	531 (8.3%)	
Baseline CD4				
Median (IQR)	389.0 (212.0, 606.0)	274.0 (142.0, 452.0)	452.0 (270.0, 660.0)	<.0001
Missing	794 (8.3%)	372 (11.6%)	422 (6.6%)	<.0001
High (>500 cells/µL)	3183 (33.1%)	581 (18.1%)	2602 (40.6%)	
Moderate (>350 to <= 500 cells/µL)	1703 (17.7%)	470 (14.6%)	1233 (19.3%)	
Low (>200 to <=350 cells/µL)	1858 (19.3%)	789 (24.5%)	1069 (16.7%)	

Very Low (<= 200 cells/µL)	2081 (21.6%)	1003 (31.2%)	1078 (16.8%)	
AIDS defining event on or prior to ABC initiation				
Yes	1924 (20.0%)	965 (30.0%)	959 (15.0%)	<.0001
No	7695 (80.0%)	2250 (70.0%)	5445 (85.0%)	

8.4. HLA-B*5701 Testing Characteristics

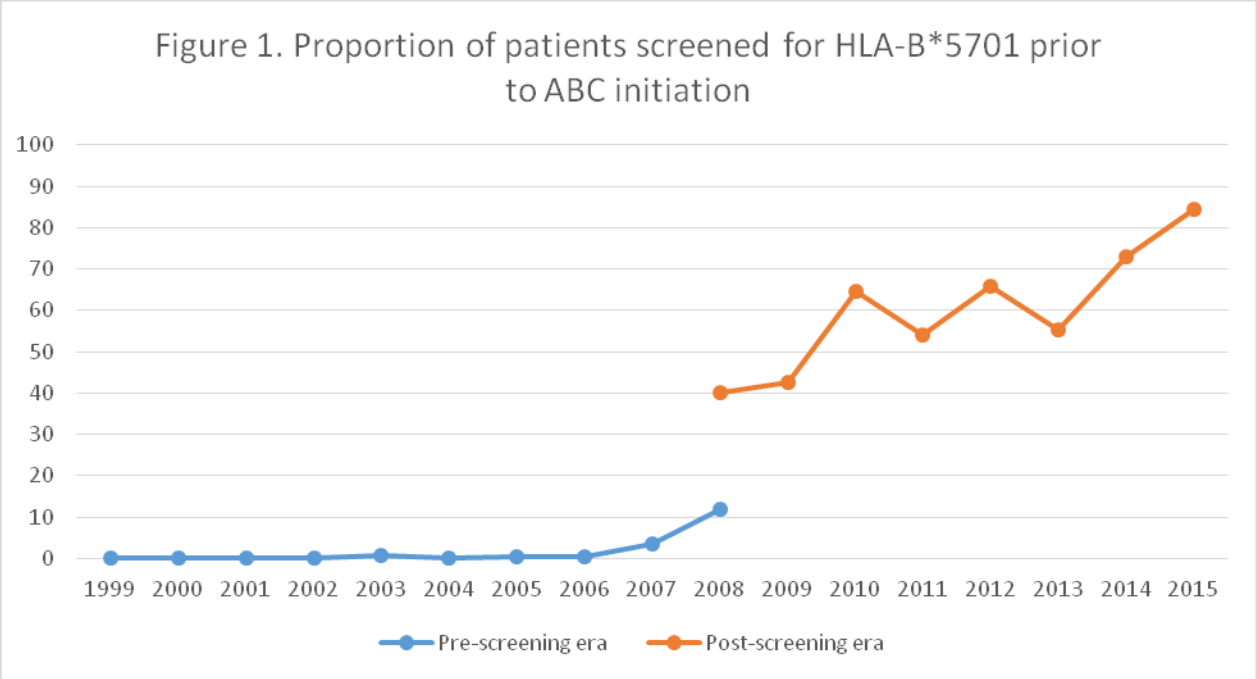
8.4.1. Table 4a: Baseline HLA-B*5701 screening characteristics for patients initiating treatment with abacavir

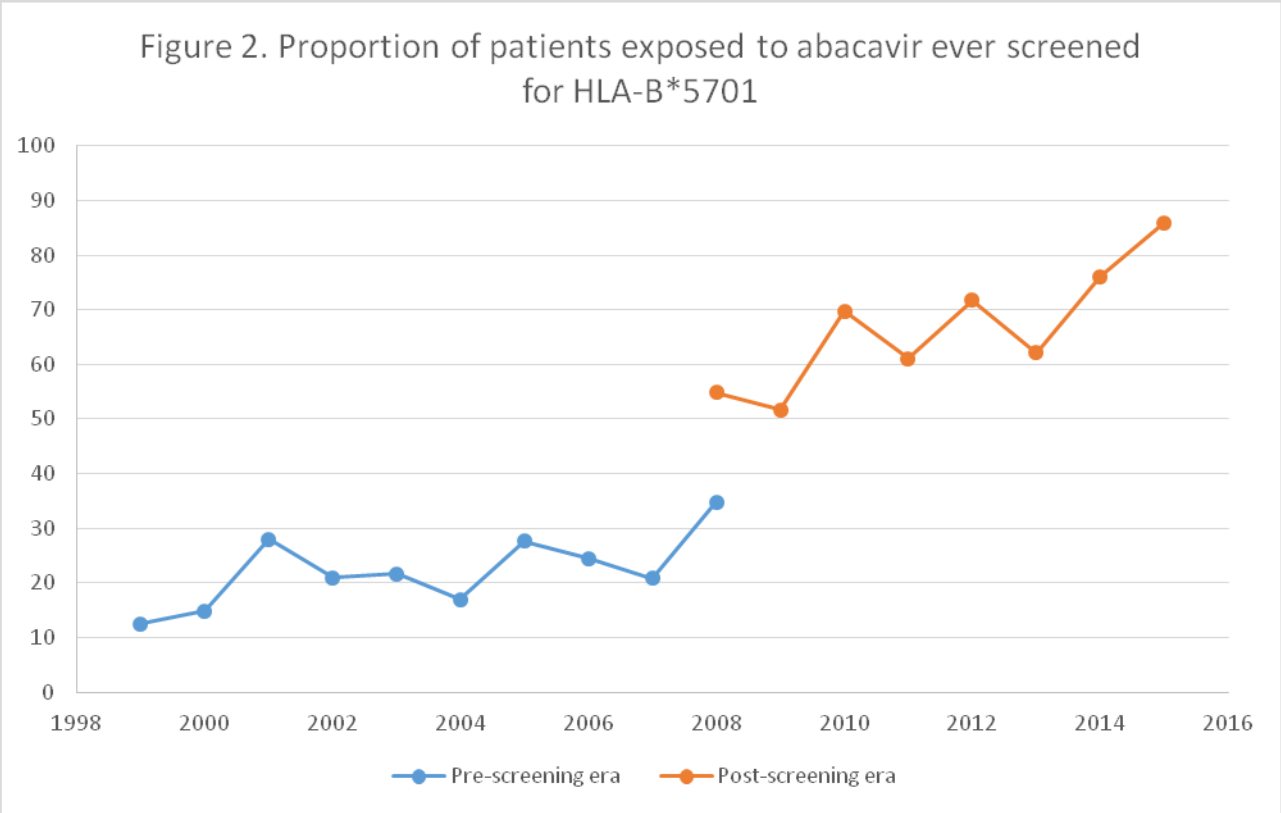
		Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Ever Screened for HLA-B*5701	Yes	768 (23.9%)	4645 (72.5%)	<.0001
Screened **before** ABC initiation	Yes	50 (6.5%)	4343 (93.5%)	<.0001
Had a positive screening test **before** ABC initiation	Yes	0 (0%)	27 (0.6%)	0.5760
Days between test and ABC start Median (IQR)	Median (IQR)	15.5 (1.0, 49.0)	44.0 (16.0, 370.0)	<.0001
Screened **after** ABC initiation	Yes	138 (18.0%)	187 (4.0%)	0.0004
Had a positive screening test **after** ABC initiation	Yes	1 (0.7%)	3 (1.6%)	0.6397
Days between ABC start and test Median (IQR)	Median (IQR)	1294.0 (488.0, 2647.0)	104.0 (28.0, 536.0)	<.0001
Screened **after** discontinuing initial ABC exposure	Yes	580 (75.5%)	115 (2.5%)	<.0001
Had a positive screening test **after** ABC initiation and discontinuation	Yes	27 (4.7%)	4 (3.5%)	0.8046

8.4.2. Table 4b: HLA-B*5701 screening by year of abacavir initiation

		Abacavir Initiations by Year N=9619	Ever Screened for HLA-B*5701 N= 5413	Screened for HLA-B*5701 before starting ABC N= 4393
Year of ABC initiation	1999	8	1 (12.5%)	0 (0.0%)
	2000	141	21 (14.9%)	0 (0.0%)
	2001	599	168 (28.0%)	1 (0.2%)
	2002	544	114 (21.0%)	1 (0.2%)
	2003	264	57 (21.6%)	2 (0.8%)
	2004	294	50 (17.0%)	0 (0.0%)
	2005	388	107 (27.6%)	1 (0.3%)
	2006	319	78 (24.5%)	1 (0.3%)
	2007	408	85 (20.8%)	14 (3.4%)
	2008	505	227 (44.9%)	132 (26.1%)
	2009	648	335 (51.7%)	276 (42.6%)
	2010	411	287 (69.8%)	266 (64.7%)
	2011	458	280 (61.1%)	248 (54.1%)
	2012	470	337 (71.7%)	309 (65.7%)
	2013	763	474 (62.1%)	423 (55.4%)
	2014	1299	988 (76.1%)	949 (73.1%)
	2015	2099	1803 (85.9%)	1769 (84.3%)
	2016	1	1 (100.0%)	1 (100.0%)

8.4.3. Figures of HLA-B*5701 screening over time





8.5. Main Analysis- Physician Adjudicated Hypersensitivity Reaction Outcomes within 6 weeks of abacavir initiation

8.5.1. Table 5a: Physician adjudicated hypersensitivity reaction events and deaths within 6 weeks of abacavir initiation

		All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Adjudicated HSR within 6 weeks of ABC initiation	Yes	88 (0.9%)	51 (1.6%)	37 (0.6%)	<.0001
Days to adjudicated HSR after ABC initiation	Median (IQR)	20.0 (12.0, 28.5)	20.0 (12.0, 29.0)	20.0 (14.0, 28.0)	0.9359

		All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Definitive or probable adjudicated HSR within 6 weeks of ABC initiation	Yes	69 (0.7%)	42 (1.3%)	27 (0.4%)	<.0001
Days to Definitive or probable HSR after ABC initiation	Median (IQR)	17.0 (10.0, 27.0)	17.0 (11.0, 27.0)	16.0 (9.0, 27.0)	0.7028
Possible deaths within 14 days of adjudicated HSR event	Yes	7 (0.1%)	1 (0.0%)	6 (0.1%)	0.4370

8.5.2. Table 5b: Physician adjudicated hypersensitivity reaction events within 6 weeks of abacavir initiation by year of abacavir initiation

		ABC Initiation By Year N=9619	All HSR events (definite, probable and possible) N= 88	Definite or probable HSR events only N= 69
Year of ABC initiation	2000	141	2 (1.4%)	2 (1.4%)
	2001	599	8 (1.3%)	7 (1.2%)
	2002	544	12 (2.2%)	10 (1.8%)
	2003	264	3 (1.1%)	2 (0.8%)
	2004	294	5 (1.7%)	4 (1.4%)
	2005	388	6 (1.5%)	4 (1.0%)
	2006	319	6 (1.9%)	5 (1.6%)
	2007	408	8 (2.0%)	7 (1.7%)
	2008	505	4 (0.8%)	4 (0.8%)

		ABC Initiation By Year N=9619	All HSR events (definite, probable and possible) N= 88	Definite or probable HSR events only N= 69
	2009	648	12 (1.9%)	5 (0.8%)
	2010	411	3 (0.7%)	2 (0.5%)
	2011	458	5 (1.1%)	3 (0.7%)
	2012	470	2 (0.4%)	2 (0.4%)
	2013	763	2 (0.3%)	2 (0.3%)
	2014	1299	6 (0.5%)	6 (0.5%)
	2015	2099	4 (0.2%)	4 (0.2%)

8.5.3. Table 5c: Incidence density rates for physician adjudicated hypersensitivity reaction within 6 weeks of abacavir initiation

	HSR cases	Person-days on abacavir	IR[1] (95% CI)[3]
Any HSR Diagnosis			
All patients	88	362,761	0.00024 cases /1 p-d (0.00020, 0.00030)
Pre-HLA*B-5701 screening era	51	129,856	0.00039 cases / 1 p-d (0.00030, 0.00052)
Post-HLA*B-5701 screening era	37	232, 905	0.00016cases/ 1 p-d (0.00012, 0.00022)
IRR[2] (95% CI)	----	----	
Post- vs. pre-screening era	----	----	**0.36 (0.23, 0.55)**
Definitive/Probable HSR Diagnosis[4]			
All patients	69	362,761	0.00019 cases/1 p-d (0.00015, 0.00024)
Pre-HLA*B-5701 screening era	42	129,856	0.00032 cases/1 p-d (0.00023, 0.00044)
Post-HLA*B-5701 screening era	27	232,905	0.00012 cases/1 p-d (0.00008, 0.00017)
IRR (95% CI)	----	----	
Post-vs. pre-screening era	----	----	**0.31 (0.19, 0.52)**

1. IR=Incidence Rate
2. IRR=Incidence Rate Ratio
3. 95% CI=95% Confidence interval
4. 95% confidence interval calculated using exact methods

8.5.4. Table 5d: HLA-B*5701 screening among patients experiencing a physician adjudicated hypersensitivity reaction event within 6 weeks of abacavir initiation

	All ABC HSR cases N= 88	HSR cases among patients starting ABC in pre-HLA-B*5701 screening period N= 51	HSR cases among patients starting ABC in post-HLA-B*5701 screening period N= 37	p-value
Physician adjudicated HSR within 6 weeks of ABC initiation	88 (100.0%)	51 (100.0%)	37 (100.0%)	-
Ever screened for HLA*B-5701	34 (38.6%)	11 (21.6%)	23 (62.2%)	0.0001
Positive for HLA*B-5701	4 (4.5%)	2 (3.9%)	2 (5.4%)	1.0000
Definitive or probable adjudicated HSR within 6 weeks of ABC initiation	69 (78.4%)	42 (82.4%)	27 (73.0%)	0.2911
Ever screened for HLA*B-5701	31 (35.2%)	10 (19.6%)	21 (56.8%)	0.0003
Positive for HLA*B-5701	4 (4.5%)	2 (3.9%)	2 (5.4%)	1.0000
Death after adjudicated HSR within 6 weeks of ABC initiation	9 (10.2%)	3 (5.9%)	6 (16.2%)	0.1579
Ever screened for HLA*B-5701	1 (1.1%)	0 (0%)	1 (2.7%)	0.4205
Positive for HLA*B-5701	0 (0%)	0 (0%)	0 (0%)	.
Death after adjudicated definite or probable HSR only within 6 weeks of ABC initiation	4 (4.5%)	1 (2.0%)	3 (8.1%)	0.3054
Ever screened for HLA*B-5701	1 (1.1%)	0 (0%)	1 (2.7%)	0.4205
Positive for HLA*B-5701	0 (0%)	0 (0%)	0 (0%)	.

Figure 3: Proportion of patients by year of ABC initiation experiencing a definite or probable HSR within 6 weeks of abacavir initiation

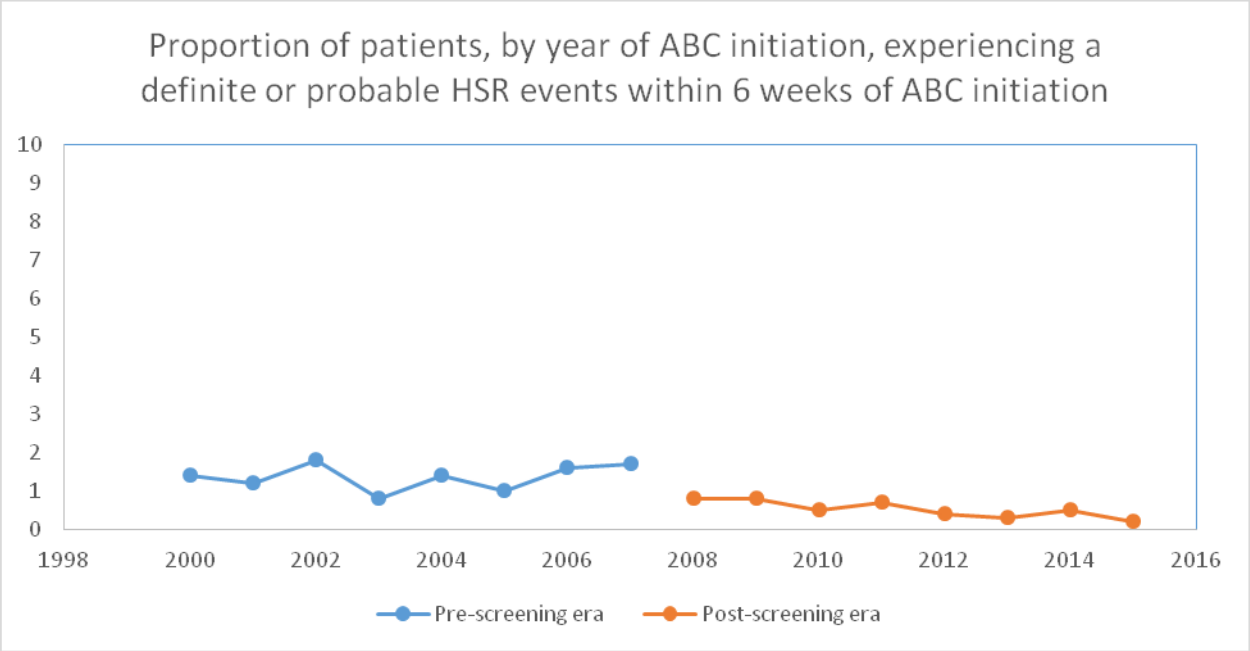
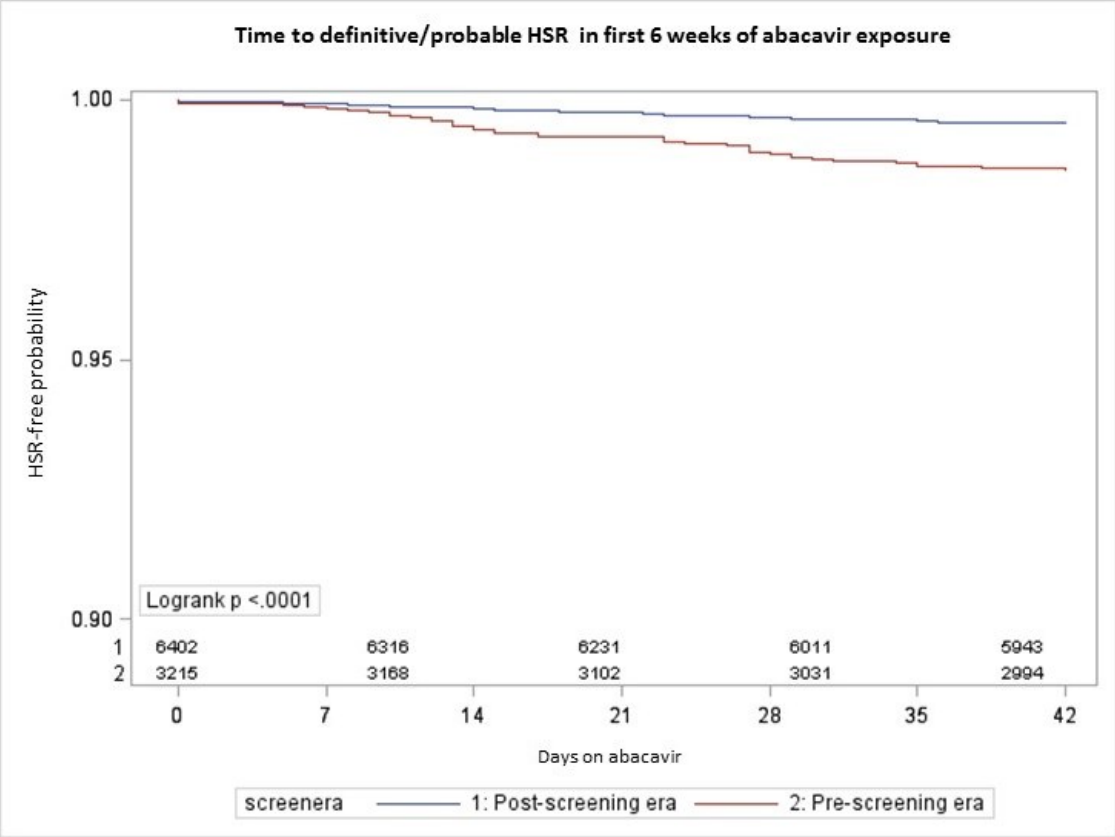


Figure 4. Kaplan-Meier plot of time to definitive/probable hypersensitivity reaction within 6 weeks of abacavir initiation, by screening era with number of subjects at risk



*Physician adjudicated definitive/probable HSR was a rare event in both eras. To better observe the curves, the y-axis has been restricted to 0.90 to 1.00.

8.6. Sensitivity Analysis- All Physician Adjudicated Hypersensitivity Reaction Outcomes Regardless of Timing

8.6.1. Table 6a: All physician adjudicated hypersensitivity reaction events and deaths among patients prescribed abacavir

		All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Any HSR diagnosis while on ABC	Yes	191 (2.0%)	90 (2.8%)	101 (1.6%)	<.0001
Days to HSR after ABC initiation	Median (IQR)	48.0 (21.0, 127.0)	35.0 (17.0, 82.0)	69.0 (27.0, 154.0)	0.0031
HSR occurring > 6 weeks after ABC initiation	Yes	102 (53.4%)	38 (42.2%)	64 (63.4%)	0.0035
Definitive or probable HSR diagnoses	Yes	88 (0.9%)	53 (1.6%)	35 (0.5%)	0.0005
Days to Definitive HSR after ABC initiation	Median (IQR)	23.0 (12.5, 35.5)	24.0 (13.0, 35.0)	22.0 (12.0, 36.0)	0.9320
Definitive HSR occurring > 6 weeks after ABC initiation	Yes	19 (21.6%)	11 (20.8%)	8 (22.9%)	0.8145
Possible deaths within 14 days of HSR diagnosis or symptom	Yes	17 (0.2%)	4 (0.1%)	13 (0.2%)	0.4523

8.6.2. Table 6b: All physician adjudicated hypersensitivity reaction events by year of initiation among patients prescribed abacavir

		ABC Initiation By Year N=9619	All HSR events (definite, probable and possible) N= 191	Definite or probable HSR events only N= 88
Year of ABC initiation	2000	141	2 (1.4%)	2 (1.4%)
	2001	599	13 (2.2%)	9 (1.5%)
	2002	544	19 (3.5%)	13 (2.4%)
	2003	264	10 (3.8%)	3 (1.1%)
	2004	294	8 (2.7%)	4 (1.4%)
	2005	388	12 (3.1%)	6 (1.5%)
	2006	319	12 (3.8%)	7 (2.2%)
	2007	408	10 (2.4%)	8 (2.0%)
	2008	505	12 (2.4%)	5 (1.0%)
	2009	648	21 (3.2%)	6 (0.9%)
	2010	411	12 (2.9%)	3 (0.7%)
	2011	458	8 (1.7%)	3 (0.6%)
	2012	470	6 (1.3%)	3 (0.6%)
	2013	763	5 (0.6%)	3 (0.4%)
	2014	1299	15 (1.1%)	7 (0.5%)
	2015	2099	26 (1.2%)	6 (0.3%)

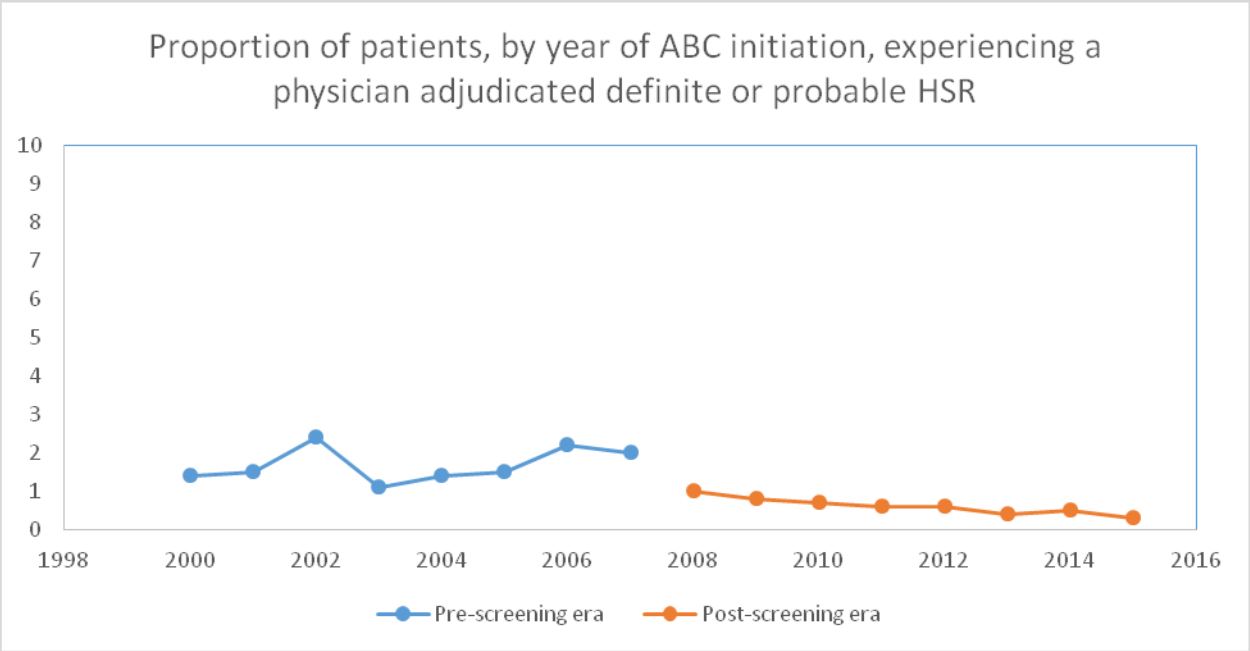
8.6.3. Table 6c: Incidence density rates for all physician adjudicated hypersensitivity reactions among patients prescribed abacavir

*Incidence density rates and comparisons could not be calculated for this sensitivity analysis because it included some very late events (approaching a year after ABC initiation) which caused the densities to become infinitesimally small.

8.6.4. Table 6d: HLA-B*5701 screening among patients experiencing a physician adjudicated hypersensitivity reaction event after being prescribed abacavir

	All ABC HSR cases N= 191	HSR cases among patients starting ABC in pre-HLA-B*5701 screening period N= 90	HSR cases among patients starting ABC in post-HLA-B*5701 screening period N= 101	p-value
Physician adjudicated HSR regardless of duration on ABC	191 (100.0%)	90 (100.0%)	101 (100.0%)	<.0001
Ever Screened for HLA*B-5701	86 (45.0%)	22 (24.4%)	64 (63.4%)	<.0001
Positive for HLA*B-5701	6 (7.0%)	3 (13.6%)	3 (4.7%)	0.1716
Definitive or probable adjudicated HSR regardless of duration on ABC	88 (46.1%)	53 (58.9%)	35 (34.7%)	0.0008
Ever Screened for HLA*B-5701	44 (50.6%)	15 (28.3%)	29 (85.3%)	<.0001
Positive for HLA*B-5701	5 (11.4%)	3 (20.0%)	2 (6.9%)	0.3187
Death after adjudicated HSR regardless of duration on ABC	17 (8.9%)	4 (4.4%)	13 (12.9%)	0.0454
Ever Screened for HLA*B-5701	1 (5.9%)	0 (0%)	1 (7.7%)	1.0000
Positive for HLA*B-5701	0 (0%)	0 (0%)	0 (0%)	.
Death after adjudicated definite or probable adjudicated HSR only regardless of duration on ABC	4 (2.1%)	1 (1.1%)	3 (3.0%)	0.6236
Ever Screend for HLA*B-5701	1 (25.0%)	0 (0%)	1 (33.3%)	1.0000
Positive for HLA*B-5701	0 (0%)	0 (0%)	0 (0%)	.

Figure 4: Proportion of patients by year of ABC initiation experiencing a physician adjudicated definite or probable HSR



8.7. Sensitivity Analysis- Hypersensitivity Reaction Outcomes by Diagnoses Only

8.7.1. Table 7a: Hypersensitivity reaction events and deaths by diagnoses among patients taking abacavir

		All ABC Initiators N= 9619	Started ABC in pre-HLA-B*5701 screening period N= 3215	Started ABC in post-HLA-B*5701 screening period N= 6404	p-value
Any HSR diagnosis while on ABC	Yes	463 (4.8%)	236 (7.3%)	227 (3.5%)	<.0001
Days to HSR after ABC initiation	Median (IQR)	249.0 (58.0, 622.0)	242.0 (44.5, 648.0)	250.0 (83.0, 579.0)	0.5731
HSR occurring > 6 weeks after ABC initiation	Yes	366 (79.0%)	178 (75.4%)	188 (82.8%)	0.0506
Definitive or probable HSR diagnoses	Yes	19 (0.2%)	18 (0.6%)	1 (0.0%)	<.0001
Days to Definitive HSR after ABC initiation	Median (IQR)	18.0 (11.0, 30.0)	20.5 (12.0, 30.0)	0.0 (0.0, 0.0)	0.1001
Definitive HSR occurring > 6 weeks after ABC initiation	Yes	4 (21.1%)	4 (22.2%)	0 (0%)	1.0000
Death within 14 days of HSR diagnosis or symptom	Yes	6 (0.1%)	3 (0.1%)	3 (0.0%)	1.0000

8.7.2. Table 7b: Hypersensitivity reaction events by diagnoses by year of abacavir initation

		ABC Initiation By Year N= 9619	All HSR events (definite, probable and possible) N= 463	Definite or probable HSR events only N= 19
Year of ABC initiation	2000	141	7 (5.0%)	0 (0.0%)
	2001	599	45 (7.5%)	5 (0.8%)
	2002	544	47 (8.6%)	4 (0.7%)
	2003	264	26 (9.8%)	1 (0.4%)
	2004	294	22 (7.5%)	1 (0.3%)
	2005	388	33 (8.5%)	4 (1.0%)
	2006	319	21 (6.6%)	2 (0.6%)
	2007	408	22 (5.4%)	1 (0.2%)
	2008	505	24 (4.7%)	0 (0.0%)
	2009	648	38 (5.9%)	0 (0.0%)
	2010	411	24 (5.8%)	0 (0.0%)
	2011	458	24 (5.2%)	0 (0.0%)
	2012	470	14 (3.0%)	0 (0.0%)
	2013	763	23 (3.0%)	0 (0.0%)
	2014	1299	41 (3.2%)	1 (0.1%)
	2015	2099	52 (2.5%)	0 (0.0%)

8.7.3. Table 7c: Incidence density rates for hypersensitivity reaction event by diagnosis

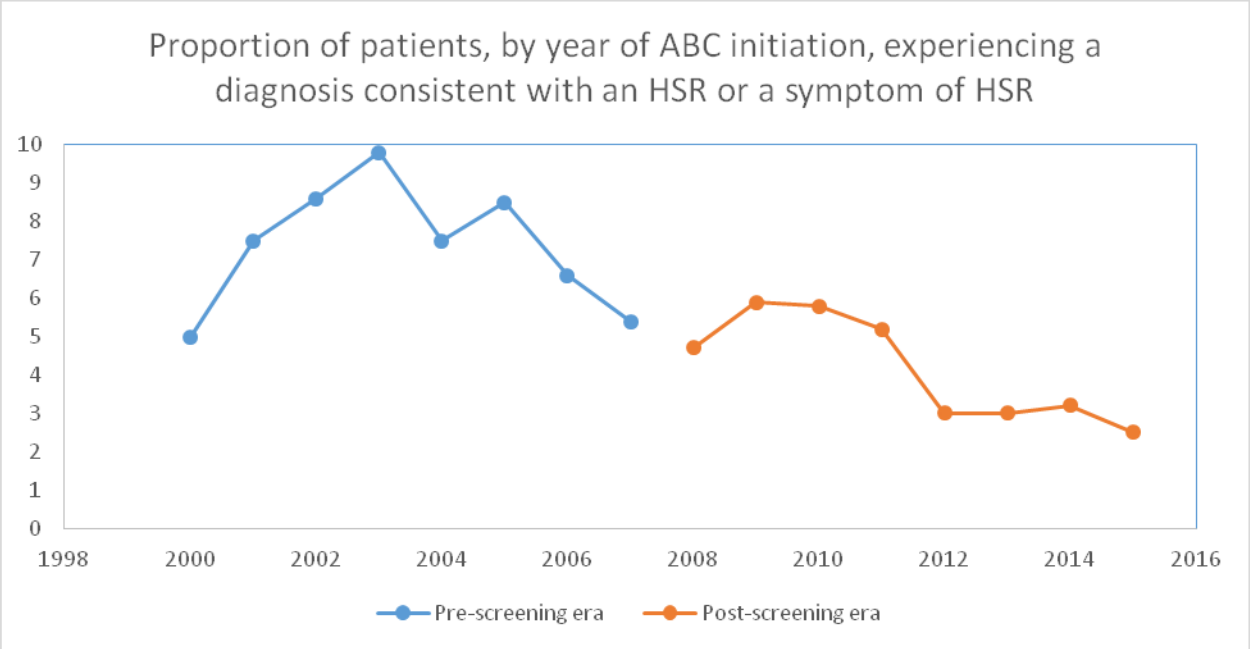
	HSR cases	Person-days on abacavir	IR[1] (95% CI)[3]
Any HSR Diagnosis			
All patients	463	7,483,804	0.062 cases /1,000 person-days on abacavir (0.056, 0.068)
Pre-HLA*B-5701 screening era	236	3,484,367	0.068 cases / 1,000 person-days on abacavir (0.060, 0.077)
Post-HLA*B-5701 screening era	227	3,999,437	0.057 cases/ 1,000 person-days on abacavir (0.050, 0.065)
IRR[2] (95% CI)	----	----	
Post- vs. pre-screening era	----	----	0.83 (0.69, 1.00)
Definitive/Probable HSR Diagnosis[4]			
All patients	19	7,455,678	0.003 cases/ 1,000 person-days on abacavir (0.002, 0.004)
Pre-HLA*B-5701 screening era	18	3,483,985	0.005 cases/1,000 person-days on abacavir (0.003, 0.008)
Post-HLA*B-5701 screening era	1	3,971,693	0.0003 cases/1,000 person-days on abacavir (0.0000, 0.0018)
IRR (95% CI)	----		
Post-vs. pre-screening era	----		***Numbers too small – Could not calculate**

1. IR=Incidence Rate
2. IRR=Incidence Rate Ratio
3. 95% CI=95% Confidence interval
4. 95% confidence interval calculated using exact methods

8.7.4. Table 7d: HLA-B*5701 screening among patients experiencing a hypersensitivity reaction event by diagnosis

	All ABC HSR cases N= 463	HSR cases among patients starting ABC in pre-HLA-B*5701 screening period N= 236	HSR cases among patients starting ABC in post-HLA-B*5701 screening period N= 227	p-value
Any HSR diagnosis while on ABC	463 (100.0%)	236 (100.0%)	227 (100.0%)	<.0001
Ever Screened for HLA*B-5701	219 (47.3%)	70 (29.7%)	149 (65.6%)	<.0001
Positive for HLA*B-5701	7 (3.2%)	3 (4.3%)	4 (2.7%)	0.6826
Definitive or probable HSR diagnoses	19 (4.1%)	18 (7.6%)	1 (0.4%)	<.0001
Ever Screened for HLA*B-5701	5 (26.3%)	5 (27.8%)	0 (0%)	1.0000
Positive for HLA*B-5701	1 (20.0%)	1 (20.0%)	0 (0%)	.

8.7.5. Figure 5. Proporation of patietns by year of ABC initiation experiencing a diagnosis consistent with an HSR or a symptom of HSR by screening period



TITLE PAGE

Information Type: ViiV Healthcare Epidemiology Study Protocol

Title:	Rates of Suspected Hypersensitivity Reaction to Abacavir and Associated Rates of HLA-B*5701 Testing

Compound Number:	GI265235
Development Phase	N/A
Effective Date:	[DD-MM-YYYY]
Subject:	Abacavir, hypersensitivity reaction, HLA-B*5701 testing
Author(s):	PPD

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1. LIST OF ABBREVIATIONS

AE	Adverse Event
GSK	GlaxoSmithKline
HIPAA	Health Insurance Portability and Accountability Act
HITECH	Health Information Technology for Economic and Clinical Health Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
MHC	Major Histocompatability Complex

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2. RESPONSIBLE PARTIES: SPONSOR INFORMATION PAGE

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SPONSOR SIGNATORY:

[Vani Vannappagari] Primary Author/ Project officer	**Date**
[Kimberly Smith] VP, Global Medical Strategy	**Date**
[Nassrin Payvandi] VP, Safety & Pharmacovigilance	**Date**

INVESTIGATOR PROTOCOL AGREEMENT PAGE

- I confirm agreement to conduct the study in compliance with the protocol.
- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name: _____

Investigator Signature	Date

3. ABSTRACT

Following the identification of a genetic link to abacavir hypersensitivity reaction, HLA-B*5701 testing entered clinical use in 2008 with the demonstration of the clinical utility of HLA screening where it was found that screening eliminated immunologically confirmed hypersensitivity reaction with a negative predictive value of 100% and a positive predictive value of 47.9%. Guidelines subsequently recommended HLA testing for all patients when considering an abacavir-containing regimen.

Objectives:

1) To describe the baseline demographic and clinical characteristics of HIV+ patients initiating an abacavir-based ART regimen.
2) To describe the annual incidence rates and cumulative frequencies of HLA-B*5701 testing before and after June 15, 2008.
3) To describe and compare the annual incidence rates and cumulative frequencies of suspected hypersensitivity reaction among abacavir-exposed patients before and after June 15, 2008.

Study Design:

An observational clinical cohort analysis utilizing prospectively collected electronic medical record (EMR) data obtained from the OPERA® Observational Database will be used to address the study objectives. The observation period will begin on January 1, 1999 (first full year post approval of Ziagen®) with study participants identified through January 1, 2016. Comparison Time Periods are 1) Pre-HLA-B*5701 screening period: January 1, 1999 to June 14, 2008, and 2) Post-HLA-B*5701 screening period: June 15, 2008 to January 1, 2016

Endpoints:

Descriptive statistics will be used to summarize baseline demographics and clinical characteristics of HIV+ patients exposed to an abacavir-containing regimen. Frequencies of HLA testing by year will be summarized. Incidence rates and cumulative frequencies of suspected hypersensitivity reaction to abacavir-containing regimens will be calculated by year of initial exposure to an abacavir-containing regimen.

4. AMENDMENTS AND UPDATES

Amendment or update no	Date	Section of study protocol	Amendment or update	Reason
<1>	<Date>	<Text>	<Text>	<Text>
<2>	<Date>	<Text>	<Text>	<Text>
<n>	<Date>	<Text>	<Text>	<Text>

5. MILESTONES

Milestone	**Planned date**
Start of data analysis	29-July-2016
Registration in the EU PAS register	28-July-2016
End of data -analysis	15-Aug-2016
Preliminary tables	19-Aug-2016
Draft report of study results	09-Sept -2016
Final report of study results	15-Oct -2016

6. BACKGROUND AND RATIONALE

6.1. Background

Abacavir sulfate, a carbocyclic 2'-deoxyguanosine nucleoside analogue, was approved by the FDA in December 1998, for the treatment of adults and children with HIV infection. The approval of abacavir was based on studies that showed improved CD4 profile and decreased plasma HIV RNA levels in patients who took abacavir in combination with other nucleoside analogues versus those who took antiretroviral regimens without abacavir.[1,2] Abacavir is converted intracellularly by enzymes, into the active compound carbovir triphosphate. This, in turn, competitively inhibits HIV reverse transcriptase and terminates proviral DNA chain extension.[3]

Originally marketed as Ziagen®, abacavir has since been co-formulated with two other nucleoside reverse transcriptase inhibitors, zidovudine and lamivudine (3TC), approved as Trizivir®, followed by co-formulations with lamivudine, approved as Epzicom® and with lamivudine and dolutegravir (DTG), approved as Triumeq®. With all formulations, abacavir is widely used to achieve viral suppression and immunologic improvement in patients with HIV infection. Factors that make abacavir a suitable choice for HIV therapy are its high oral bioavailability (geometric mean of absolute bioavailability is 83%), no significant effect of food on the extent of absorption, pharmacokinetics that support once daily dosing, good central nervous system penetration, no significant drug interactions, and slow development of drug-resistant mutants.[4-8]

Early phase I/II trials with abacavir indicated the occurrence of side effects like headache, gastrointestinal disturbances, rash, malaise, fatigue and asthenia. Like many antiretroviral drugs, abacavir is metabolized by cytochrome P450 in the liver. Therefore, as with other nucleoside analogues, patients who take abacavir are susceptible to lactic acidosis, hepatomegaly and steatosis.[9] Among nucleoside analogues, abacavir is believed to have a lower propensity for causing mitochondrial toxicity. Studies show that switching patients with symptomatic hyperlactatemia or lactic acidosis from stavudine and/or didanosine to abacavir and lamivudine result in less potent levels of hyperlactatemia.[10]

Hypersensitivity is the term used for an extreme form of adaptive immune response. Such responses occur when the immune system reacts inappropriately to certain antigens, and may lead to inflammatory reactions and tissue damage.[11] There are four types of hypersensitivity reactions – I, II, III and IV. Type I hypersensitivity is mediated by immunoglobulin E (IgE), leading to the release of pharmacological mediators which produce an acute inflammatory reaction. Type II hypersensitivity is antibody-dependent (IgG or IgM) and occurs when antibodies bind to self or foreign antigens on cells, causing phagocytosis, killer cell activity or complement-mediated lysis. Type III hypersensitivity develops when large immune complexes cannot be cleared from the reticuloendothelial system. Type IV or delayed type hypersensitivity (DTH) occurs when antigens are trapped in a macrophage and cannot be cleared. As a result, cytokines are released and these mediate a range of inflammatory responses.

The exact mechanism of abacavir hypersensitivity reactions is not clearly understood, although studies suggest the involvement of T-cells and the cytokines interferon-gamma (IFN-γ) and interleukin-4 (IL-4).[12,13] Hypersensitivity to abacavir is a multi-organ syndrome characterized by a sign or symptom in two or more of the following categories:
Group 1: Fever
Group 2: Rash
Group 3: Gastrointestinal (nausea, vomiting, diarrhea or abdominal pain)
Group 4: Constitutional (generalized malaise, fatigue, aches)
Group 5: Respiratory (dyspnea, cough, pharyngitis)

In a review of 9 clinical trials conducted between November 1999 and January 2002 involving 2,670 patients, 8% (range 2-9%) of patients prescribed abacavir reported a suspected hypersensitivity reaction. The median time to onset was 9 days, with 89% presenting symptoms within the first 6 weeks of starting the drug. The vast majority of patients (95%) presented symptoms from two or more of the groups that are described above.[26]

Other symptoms of hypersensitivity include lethargy, myolysis, edema, abnormal chest X-ray, paresthesia, liver failure, renal failure, hypotension, adult respiratory distress syndrome, respiratory failure and death. Reports of anaphylaxis with initial and re-challenge exposure to abacavir have been documented.[14-17] There have also been isolated case reports of unusual symptoms like anorexia, peri-tonsillar abscess, agranulocytosis, lip ulcers and neuropsychiatric symptoms like night sweats, depression, and auditory hallucinations.[18-20] A retrospective review of data from 200,000 patients who received abacavir through clinical trials or by prescription initially identified a total of 1,803 cases of suspected hypersensitivity to the drug. Upon further review of these cases, the

calculated incidence rate in the clinical trials was determined to be 4.3%. The mortality rate in patients who received abacavir in clinical trials was 0.03%.[21]

Barring rare hypersensitivity reactions with fatal outcomes, in general, the symptoms are reversed after the discontinuation of abacavir. However, hypersensitivity reaction is much more severe and more frequently lethal in patients who, after the resolution of initial symptoms, are reintroduced to abacavir. Following a diagnosis of hypersensitivity, patients must not take abacavir again. Restarting the drug following a hypersensitivity reaction has resulted in cases of life-threatening hypotension and fatal reactions. Additionally, there have been reports of individuals who developed re-challenge hypersensitivity to abacavir after having been asymptomatic following initial use of the drug as well.[15,22] Therefore, it is recommended that all patients receiving abacavir be monitored closely for signs of a hypersensitivity reaction, especially in the initial weeks of treatment.[23]

Early studies examining the demographic and clinical predictors of hypersensitivity found higher risks for white race, female gender, elevated baseline CD8 and lower risks for antiretroviral treatment and African American descent.[24-27] Genetic susceptibility factors have been suggested because of the occurrence of the reaction in a small sub-population of patients receiving abacavir, familial disposition, the low incidence of the reaction in patients of African American origin and involvement of the major histocompatibility complex (MHC) alleles in other similar multi-organ hypersensitivity reactions.[28,29] Later studies have found an association between abacavir hypersensitivity and specific human leukocyte antigen (HLA) alleles.[30]

Following the identification of a genetic link to abacavir hypersensitivity reaction, HLA-B*5701 testing entered clinical use in 2008 with the demonstration of the clinical utility of HLA screening where it was found that screening eliminated immunologically confirmed hypersensitivity reaction with a negative predictive value of 100% and a positive predictive value of 47.9%.[30] Guidelines subsequently recommended HLA testing for all patients when considering an abacavir-containing regimen.

6.2. Rationale

With studies showing a negative predictive value of HLA-B*5701 of 100%, treatment guidelines were adjusted after the genetic link was identified and a genetic test became available in 2008. This analysis will assess the use of HLA testing in the general HIV practice setting along with the rates of suspected hypersensitivity reaction due to abacavir in the pre-testing era compared to the post-testing era.

7. RESEARCH QUESTION AND OBJECTIVE(S)

1) To describe the baseline demographic and clinical characteristics of HIV+ patients initiating an abacavir-based antiretroviral therapy (ART) regimen.
2) To describe the annual incidence rates and cumulative frequencies of HLA-B*5701 testing before and after June 15, 2008.

3) To describe and compare the annual incidence rates and cumulative frequencies of suspected hypersensitivity reaction among abacavir-exposed patients before and after June 15, 2008.

8. RESEARCH METHODS

8.1. Study Design

An observational clinical cohort analysis utilizing prospectively collected electronic medical record (EMR) data obtained from the OPERA® Observational Database will be used to address the study objectives.

Period of Observation:

The observation period will begin on January 1, 1999 (first full year post approval of Ziagen®) with study participants identified through January 1, 2016.

Comparison Time Periods:

Pre-HLA-B*5701 screening period: January 1, 1999 to June 14, 2008

Post-HLA-B*5701 screening period: June 15, 2008 to January 1, 2016

8.2. Study Population and Setting

The study sample will be identified from the OPERA Observational Database for analysis. HIV-1 positive patients initiating abacavir-containing treatment for the first time between 1/1/1999 and 1/1/2016 will be included in the study sample if they meet the following inclusion criteria:

1) At least 13 years of age at the index date.
2) Continuous clinical activity in the year prior to abacavir initiation, defined as at least one clinic visit.
3) Continuous clinical activity in the year following abacavir initiation, defined as at least one clinical contact (visit or telephone contact).

Follow-up Period: Patients will be observed from their initiation of abacavir until the first of the following censoring events: a) discontinuation of abacavir, b) cessation of continuous clinical activity, c) death or d) study end (July 31, 2016). Patients failing to meet the continuous clinical activity requirement will be censored 12 months after their last contact.

8.3. Variables

8.3.1. Exposure definitions

- First exposure to abacavir (Ziagen, Trizivir, Epzicom, Triumeq)

8.3.2. Outcome definitions

- Diagnosis of suspected hypersensitivity reaction to abacavir (HSR)
- Documentation of HLA-B*5701 testing and timing of the test (before starting ABC containing regimen or after)
- Exposure to abacavir post positive HLA testing

8.3.3. Confounders and effect modifiers

Confounding may occur with the use of other ART that causes similar symptoms (e.g. nevirapine and rash) or from other illnesses that could be confused with these symptoms (e.g. influenza season). Effect modification is anticipated to be minimal for this particular analysis insofar as the awareness of HSR associated with abacavir was high in both the pre-2008 and post-2008 periods. Evidence of the level of awareness of HSR with abacavir, especially with potential re-challenges, is available from the Trizivir Epidemiology Program, a post-approval study requirement. In this study, re-challenges with abacavir post HSR diagnoses were minimal, suggesting a high level of awareness of the diagnostic algorithm for HSR and the contraindication of abacavir re-challenge. It is possible that, post-2008, prescriber comfort with abacavir increased due to the availability of HLA testing and its 100% negative predictive value.

8.4. Data sources

The OPERA® (Observational Pharmaco-Epidemiology Research & Analysis) database and research network is a multi-site observational database built from the complete patient health records managed in Electronic Health Record (EHR) systems throughout the U.S.

In total, there are more than 2.5 million documented prospective visits in the EHR systems for HIV+ patients and 2.6 million prescriptions written for ART medications. The average years of follow-up (years of documenting patient visits prospectively in the EHR) for patients in OPERA is 4.2 years and there are 5,479 HIV+ patients who have nine years or more of follow-up.

In addition to HIV treatment, OPERA captures the diagnosis and treatment of co-morbid conditions and diagnoses of HIV negative patients. Epividian analyses other serious comorbid conditions such as Hepatitis C with about 8% of HIV+ patients in OPERA co-infected.

8.5. Study size

As of March 2016, 17,253 patients in OPERA have had an HLA-B*5701 test performed in OPERA. In total, 16,676 patients have been exposed to abacavir in one or more of the various formulations (with a cumulative number of treatment days of 17.2 million). Of these 16,676 patients, 6,638 started an abacavir-containing regimen prior to April 1, 2008.

8.6. Data management

8.6.1. Data handling conventions

Epividian utilizes a number of proprietary algorithms to sort, classify, and aggregate the data pulled from the participating clinics' EHR systems. The process includes automated classification of clinical terms into common clinical terms with review by trained medical staff. The standardization of the data to common terms and application of the Epividian knowledge base are key process steps in gathering data from multiple heterogeneous EHR systems and databases from many locations into a single, homogenous OPERA database for conducting research and commercial analyses. The patient health data gathered, classified and aggregated includes complete medical history & social history, visit dates, vital signs, lab orders and results, medications, problems & diagnoses, and procedures.

Epividian has developed rigorous data management processes that include both automated and manual quality checks. Data quality methods include common techniques such as:

- Detection and reporting of outliers that lead to correction, acceptance, or exclusion of observations; these can include a medical review.
- Detection of potentially missing data (e.g. a patient taking ART medications with no history of HIV infection to determine whether the use was prophylactic or treatment for infection diagnosed elsewhere).
- Data completion using multiple observations and sources (e.g. using diagnoses codes, free text, past medical history, etc. to determine if patient is naive to HIV therapy).
- Detection of observations that are known to be (or likely to be) mutually exclusive for a patient (e.g. record of medications that are typically not administered concurrently).

8.6.2. Resourcing needs

Addressed in contract.

8.6.3. Timings of Assessment during follow-up

Not applicable.

8.7. Data analysis

8.7.1. Essential analysis

Descriptive analyses:

> Demographics: Age, sex, race (African American/non-African American), ethnicity (Hispanic/non-Hispanic), risk of infection (MSM/other), and geographic region

Clinical: ABC formulation (Ziagen/ Trizivir/ Epzicom/ Triumeq), therapy experience (naïve/experienced), concomitant nevirapine use (yes/no), flu season administration (Dec-Mar/other), time period (pre-HLA testing/ post-HLA testing), HLA testing status (yes/no), HLA test results (positive/negative), time from HLA testing to first abacavir exposure. Note: HSR events most commonly occur within 2 weeks of abacavir initiation, events occurring after 6 weeks of initiation of an Abacavir containing regimen will be tabulated separately, as these are often not confirmed as HSR. Time to HSR event tables and Kaplan-Meyer curves will be used to assess the average and median time for the occurrence of suspected HSR.

Multivariable analyses:

Rates of suspected HSR diagnoses by HLA time period.

8.7.2. Exploratory analysis

Assess abacavir exposure rates prior to HLA testing completion in post-06/15/2008 era.

8.7.3. General considerations for data analyses

Missing or incomplete data are not uncommon in the observational setting in which measures are collected through routine clinical care rather than on a set schedule dictated by a protocol associated with a clinical trial.[31] In this setting, data may not be missing at random which can lead to a biased measure of association and overly precise confidence intervals if only those observations with complete data are used in the analysis.[32]

Patients lost to follow up are flagged and their follow up time censored. Sensitivity analyses can be used to elucidate the importance of their contribution to any conclusions.

Stockpiling of medication through incomplete adherence can result in gaps in the medication record. These will be handled by collapsing the medication record across gaps of less than 30 days in which the patient returns to the same medication. A collapse will not occur if it coincides with a suspected hypersensitivity event to allow observation of re-challenge events.

8.8. Quality control and Quality Assurance

Epividian has working practices & procedures governing the use of observational data, the development of analysis specifications and plans, the development of analytical programming, the analytical quality assurance process and the scientific review of reports as well as clinical advisory charters for the clinical review of output intended for public domain. Working practices for the development of analysis specifications include basic identifying information, background material, relevant definitions of key study variables, population definitions, baseline definitions, specific requirements for dataset creation, statistical requirements such as eligibility criteria, exposures, outcomes and model fitting. Working practices for programming include naming conventions, proper code documentation and commentary, content, appearance, efficiencies (i.e. use of macros), and organization of output, maintainability and generalizability. Working practices for programming quality assurance include self-reviews of observational counts, missing

data values, many-to-many merges, variable formatting, numeric-character & character-numeric conversions, uninitialized variables, unresolved macro references, report completeness and report-to-specification correspondence, and system errors and logs. The quality assurance team review may include small sample spot-checking, coding log reviews, complete coding review, selected observations from intermediary dataset reviews, and/or independent programming to reproduce the results. Documentation of non-public domain reports includes market, scientific, statistical, and clinical review. Documentation of scientific protocols, reports and manuscripts intended for public domain follows two sequential steps: an internal-to-Epividian epidemiological, statistical, and clinical review, followed by a clinical/epidemiological external advisory board review.

8.9. Limitations of the research methods

With approximately 7% of the HIV population that is linked to care in the OPERA database (per the CDC estimates), OPERA can provide detailed information on a large portion of the HIV population in the U.S. Even so, issues confronting population-level assessments include such aspects as differential medical care by practice size and specialty, academic and research orientation of the health care practitioner, ethnic-based & gender-based attitudes and geographic regional health care practices. OPERA clinical data is collected at point-of-care and is subject to the record-keeping practices of each healthcare provider and the standards of each clinic or organization. Patients may see multiple physician practices for various conditions, which may result in incomplete case ascertainment. Data is collected for the medical management of patients and is not directly intended for research purposes, but rather for the care and management of individual patients and patient populations.

8.9.1. Study closure/uninterpretability of results

Closure is at report submission.

8.10. Other aspects

The OPERA® Epidemiological and Clinical Advisory Board provides all methodological and clinical oversight.

9. PROTECTION OF HUMAN SUBJECTS

Clinical information is aggregated into the OPERA® Database following the guidelines of HIPAA and HITECH. Data aggregation occurs via a secure and encrypted connection with security and confidentiality maintained through Epividian's validated de-identification algorithms with regular and routine statistical audits of the de-identification process.

9.1. Ethical approval and subject consent

Business Associate Agreements (BAA) in place between Epividian and all medical practices govern, following the guidelines established in HIPAA and HITECH, the encryption, transportation, aggregation, de-identification and use of all clinical data in the

OPERA® Database. All medical practices are responsible for obtaining proper HIPAA consent for their patients. With BAA's in place, a separate informed consent for each individual, non-interventional study is not required.

9.2. Subject confidentiality

All clinical data is de-identified as per HIPAA and HITECH. The OPERA® Clinical Advisory Board provides clinical and methodological review & oversight.

All clinical data is de-identified as per HIPAA and HITECH in OPERA® with all reports submitted at the aggregated population level. No personally identifiable information is available in the OPERA® Database.

10. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

The study design is to analyse the patient level information recorded in the OPERA database from electronic health records in an aggregate manner. Reporting of adverse events by Epividian to competent authorities is not applicable as the healthcare information used in this study will not contain physician attribution of adverse event causality to any medicinal product.

11. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

11.1. Target Audience

Health care practitioners, regulatory authorities

11.2. Study reporting and publications

Final report to be submitted to sponsor. Study results will be submitted to a peer reviewed journal.

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