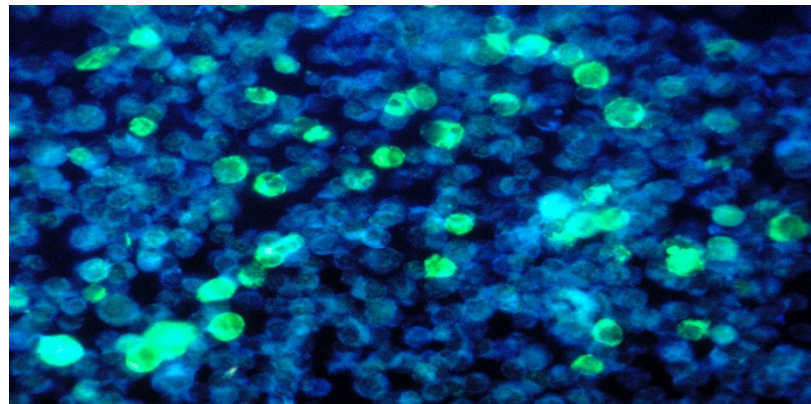


Draft RoC Monograph Epstein-Barr Virus



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Outline

Properties

Detection

Transmission

Exposure

Mechanistic information

Human cancer studies

Preliminary level of evidence conclusions



- Also known as human herpesvirus 4 (HHV4)
- An enveloped dsDNA virus in the gammaherpesvirus subfamily, consisting of two major types (EBV-1 and EBV-2)
- EBV predominantly enters the host through contact with saliva and infects B cells in the tonsils
- Lifelong latent infection in memory B cells that is refractory to immune recognition
- Activated EBV transcription programs during latency mimic B cell proliferation and survival and in some cases result in cancer



EBV Detection and Transmission

- Detection
 - Anti-EBV antibodies in the serum
 - e.g. VCA, EA, and EBNA antibodies (IgG, IgM, or IgA)
 - EBV DNA or RNA in peripheral white blood cells or tumor tissue; DNA in plasma or serum
- Transmission
 - EBV is primarily transmitted via saliva
 - EBV in peripheral blood suggests transmission via blood is possible
 - Transmission among transfusion and organ recipients has been reported
 - Infected cells (primarily resting memory B cells) provide a permanent reservoir for EBV (Latency 0)



- Significant exposure in the United States
 - U.S. seroprevalence (2009-2010) 50% in 6-8 year olds and 89% in 18-19 year olds
 - Infection rate worldwide is high, exceeding 90%
 - The age of infection varies geographically
- EBV symptoms
 - EBV infection is asymptomatic in most individuals
 - Infection is life-long
 - Related diseases
 - EBV can lead to infectious mononucleosis, cancer
 - No vaccine

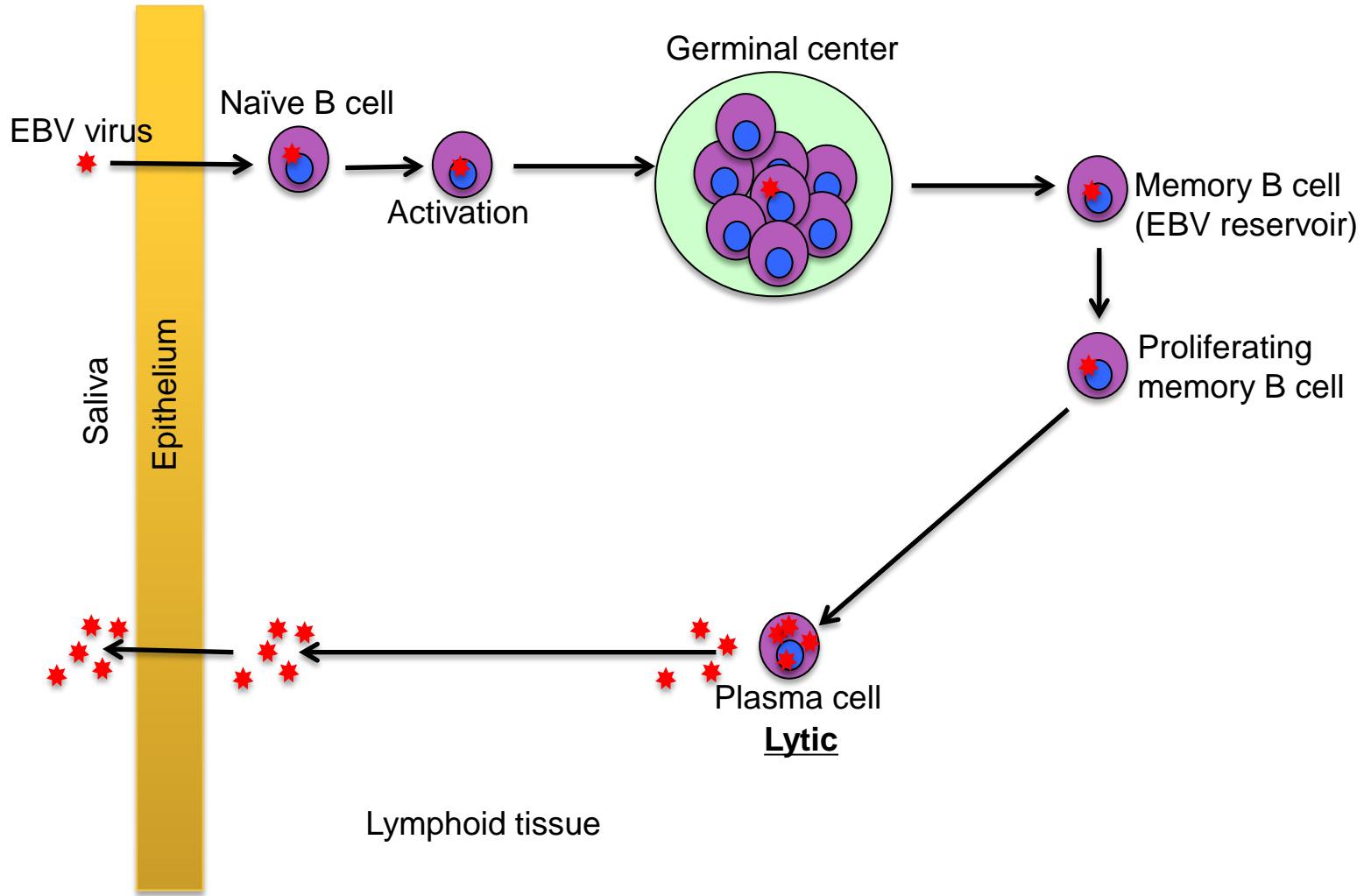


ORoC Evaluated 7 Endpoints

- Lymphomas
 - Burkitt lymphoma
 - Endemic
 - Sporadic
 - Hodgkin lymphoma
 - Immunosuppression-related non-Hodgkin lymphoma
 - NK/T-cell lymphoma (nasal type)
- Epithelial cancers
 - Nasopharyngeal carcinoma
 - Gastric cancer
 - Lymphoepithelial cancer of the salivary gland

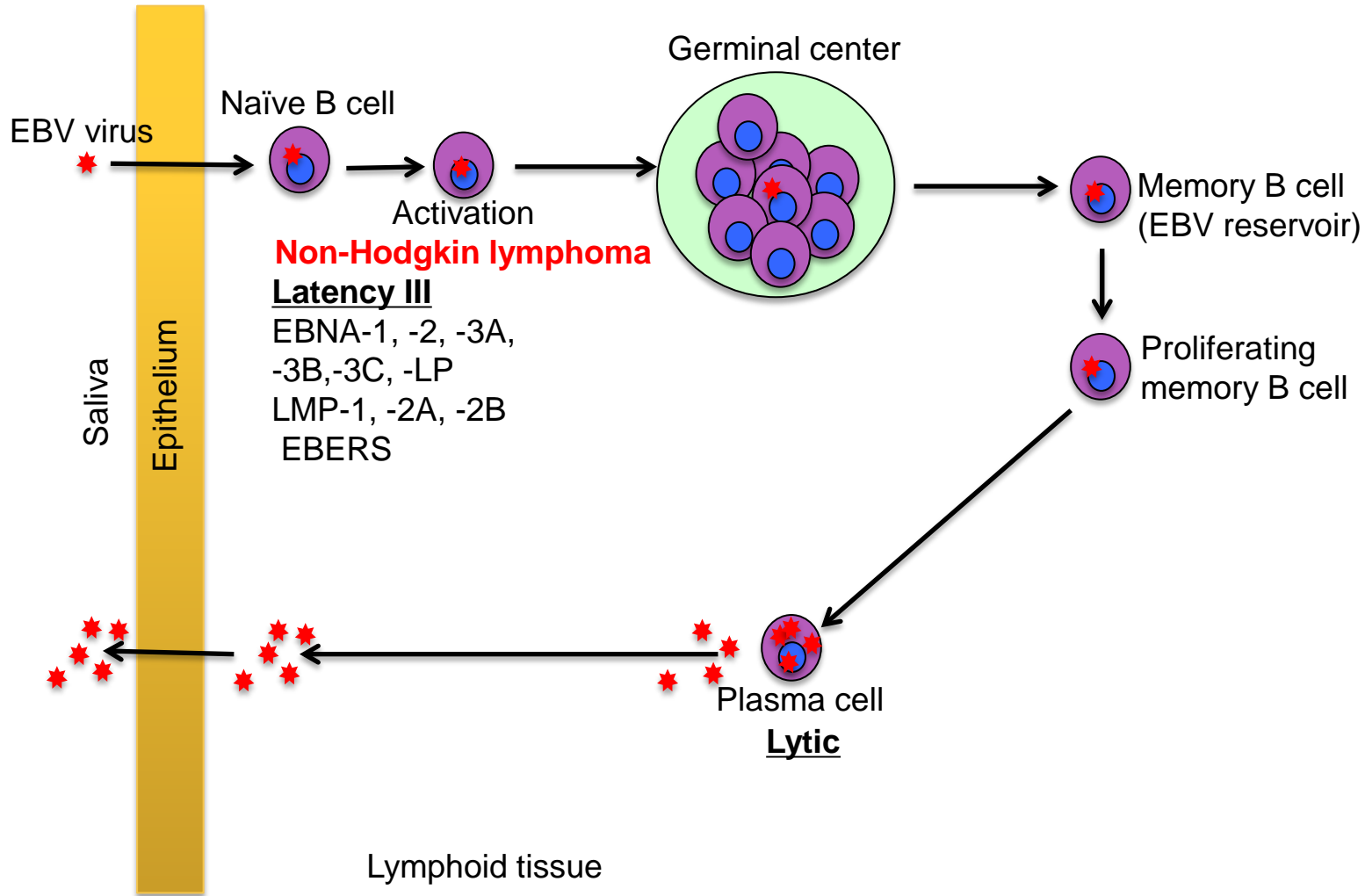


EBV Life Cycle, Lymphomas and Latency Proteins



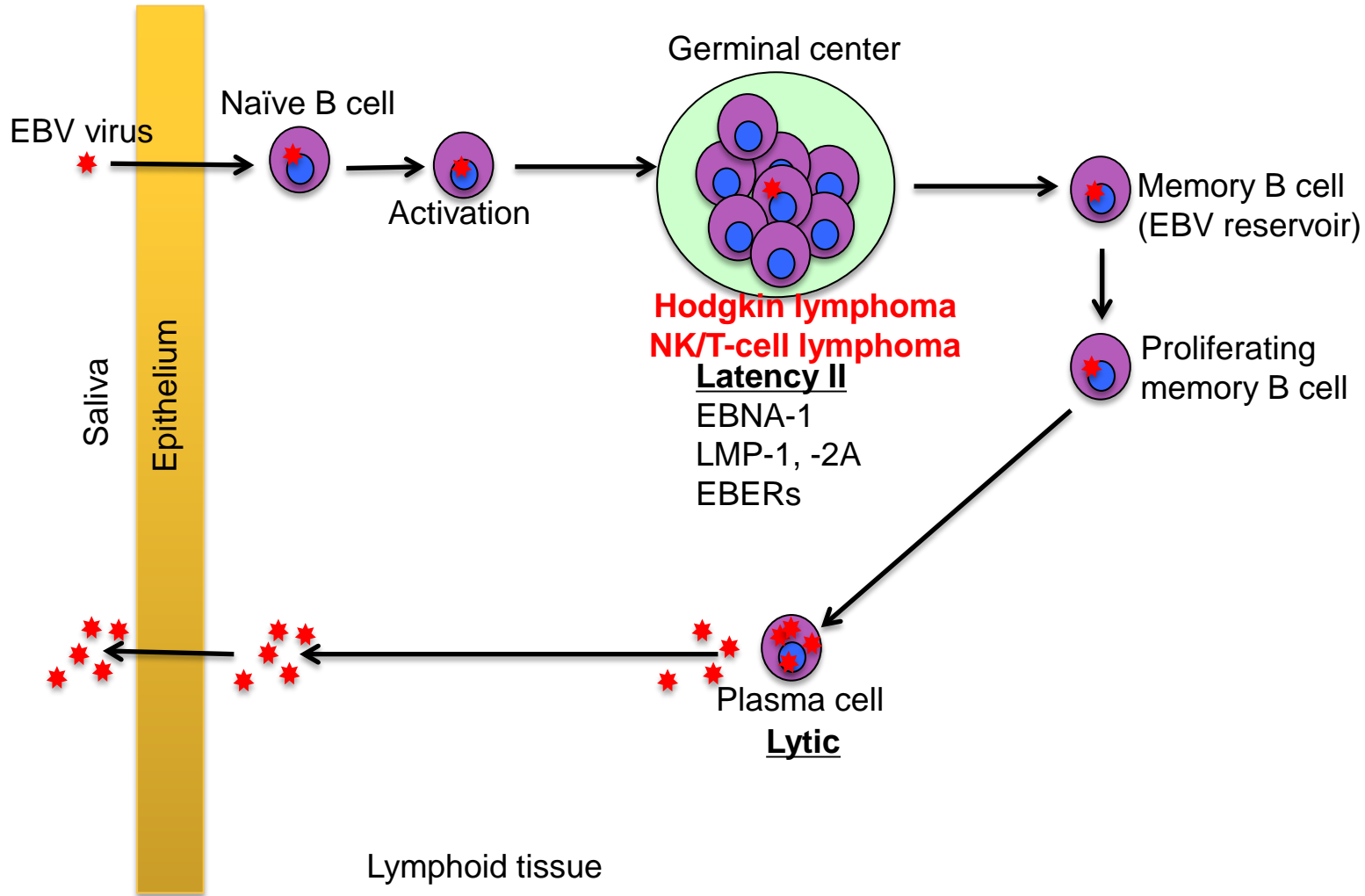


EBV Life Cycle, Lymphomas and Latency Proteins



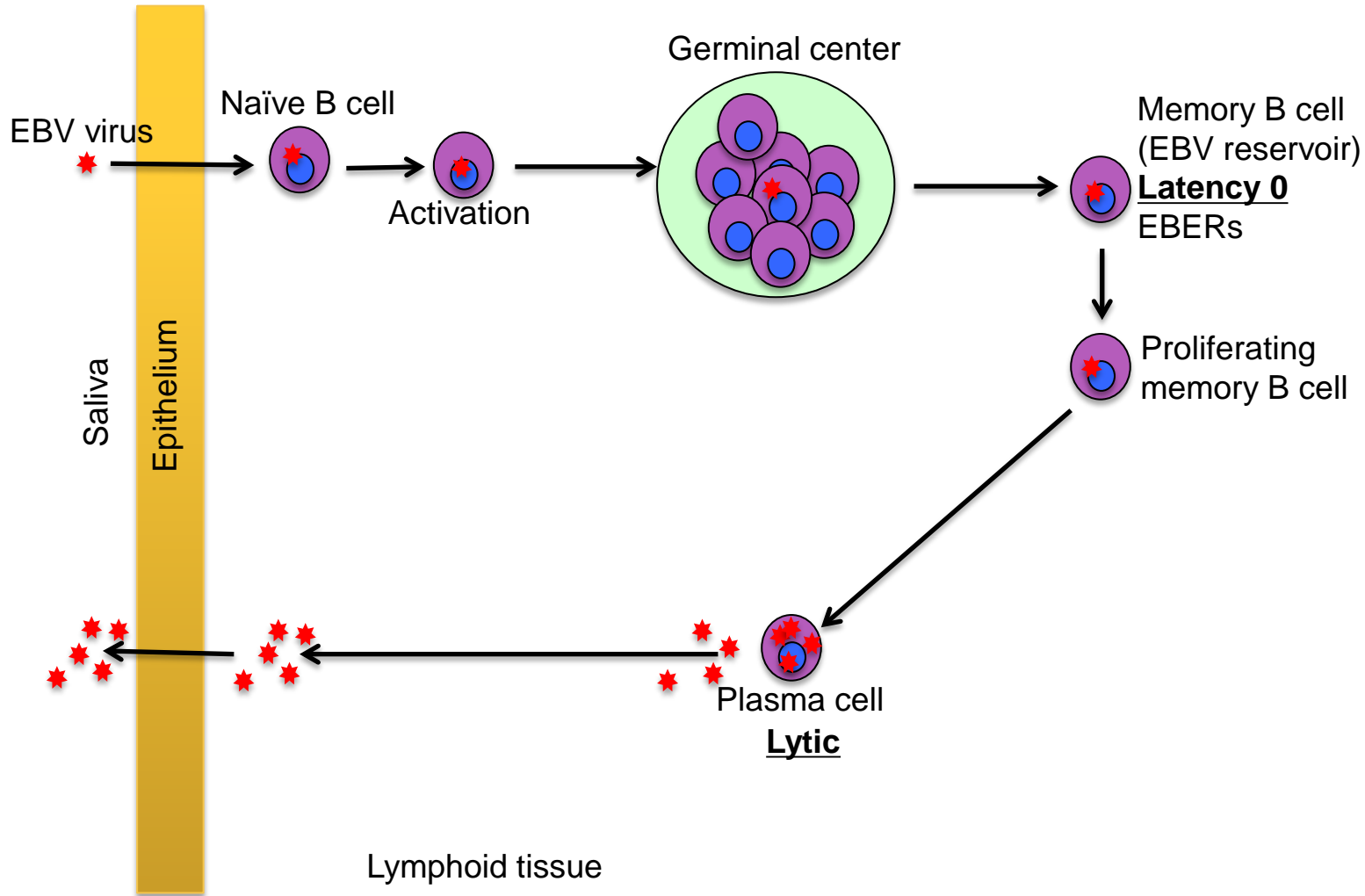


EBV Life Cycle, Lymphomas and Latency Proteins



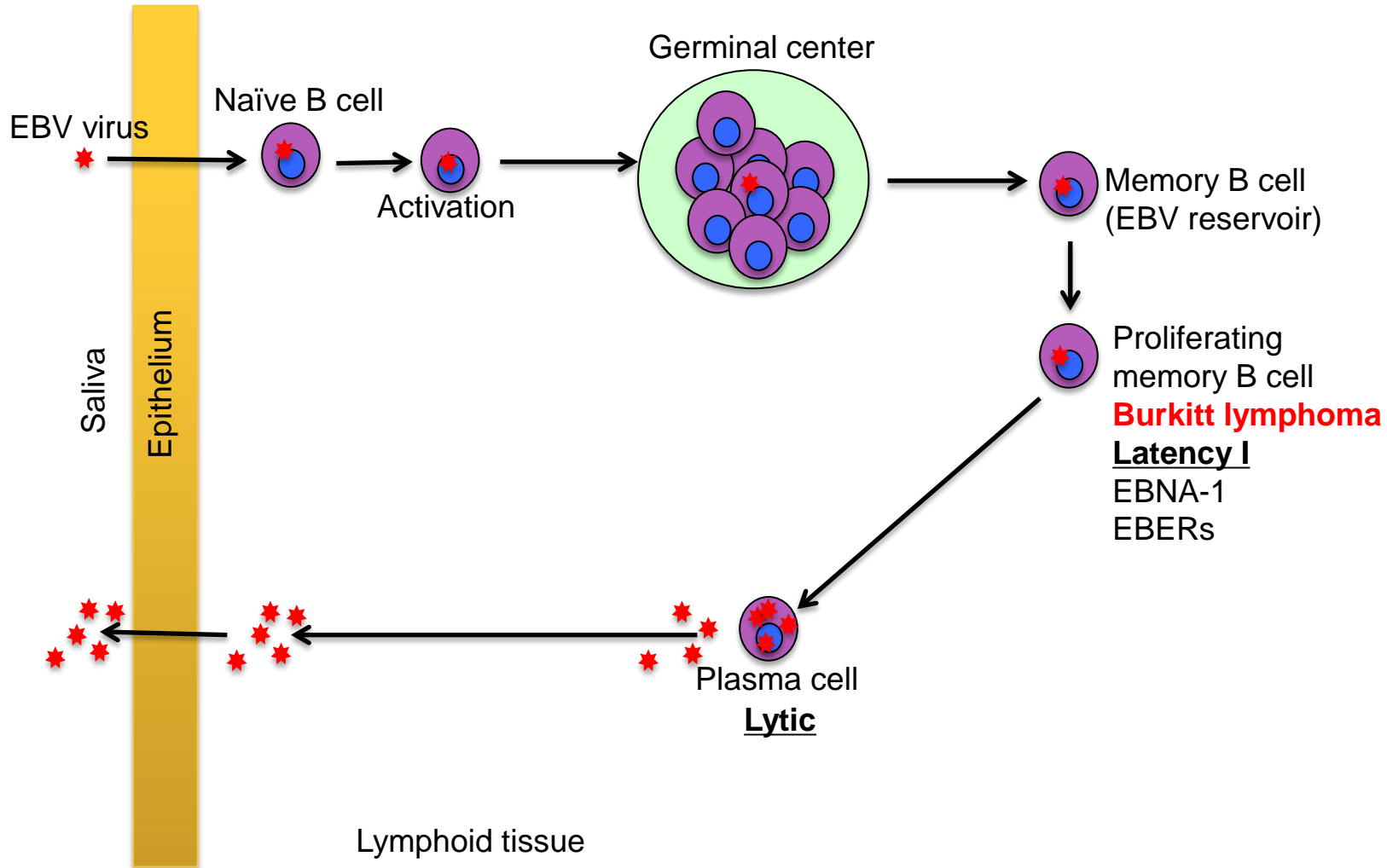


EBV Life Cycle, Lymphomas and Latency Proteins





EBV Life Cycle, Lymphomas and Latency Proteins





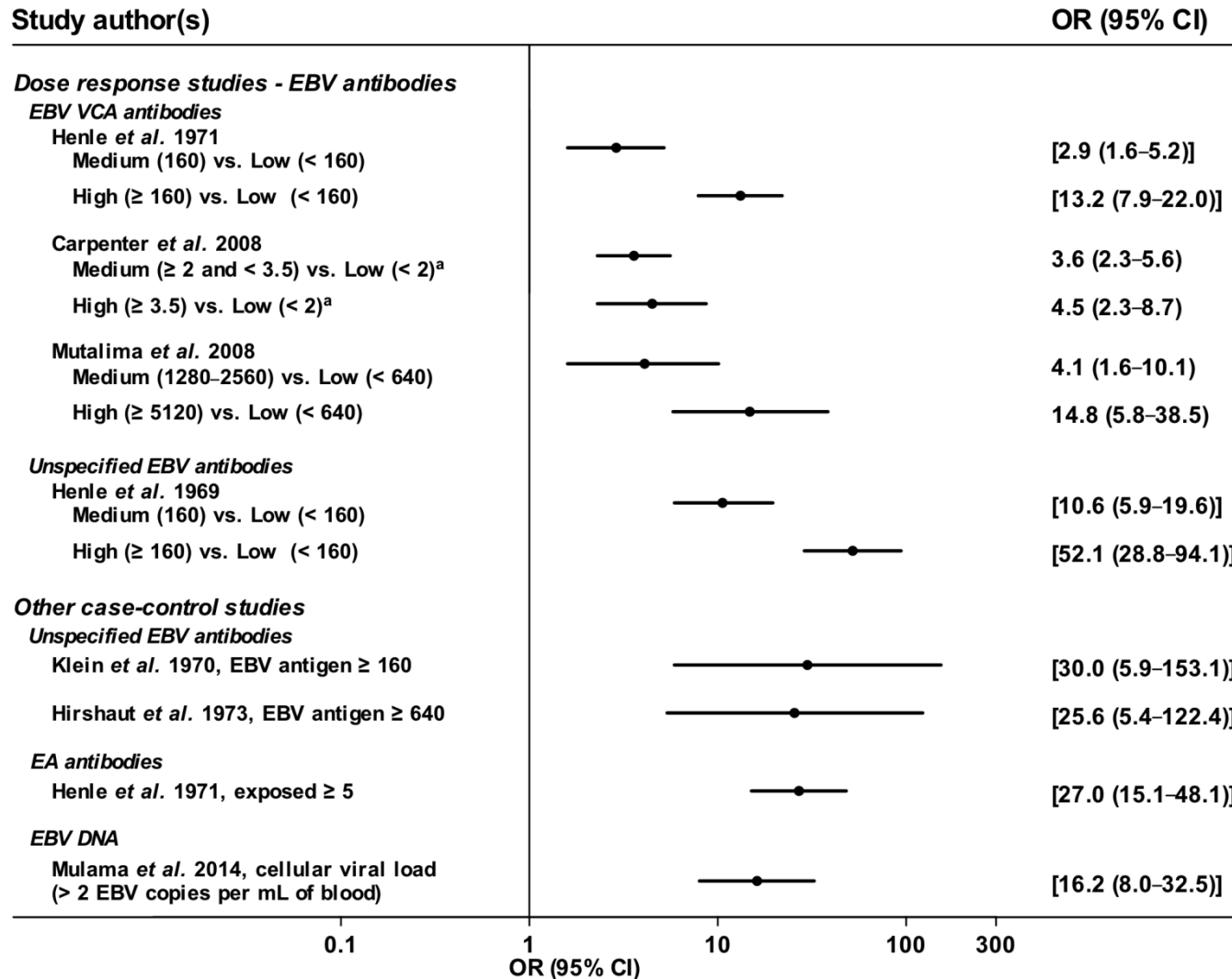
Lymphomas

- Burkitt Lymphoma
 - Endemic
 - Sporadic
- Hodgkin Lymphoma
- NK/T-cell Lymphoma (Nasal Type)
- Immunosuppression-related Non-Hodgkin Lymphoma



Burkitt Lymphoma (Endemic)

Serological case-control studies of endemic Burkitt lymphoma and EBV





Burkitt Lymphoma (Endemic)

Sufficient level of evidence for endemic Burkitt lymphoma

Epidemiology

Studies with positive associations
or dose-response

EBV antibodies or DNA: 7/7 case-control (993 cases) & 1
cohort study.

All statistically significant; high RR/ORs.

Dose-response with viral titer in cohort study and several case-
control studies.

Human tissue

Clonality

Monoclonal

% EBV-infected tumors

95%

EBV protein expression

EBNA-1



Burkitt Lymphoma (Sporadic)

Limited level of evidence for sporadic Burkitt lymphoma

Epidemiology

Studies with positive associations
or dose-response

EBV antibodies: 4/5 case-control studies (113 cases).

Most not significant; moderate ORs

Human tissue

Clonality

NA

% EBV-infected tumors

20%

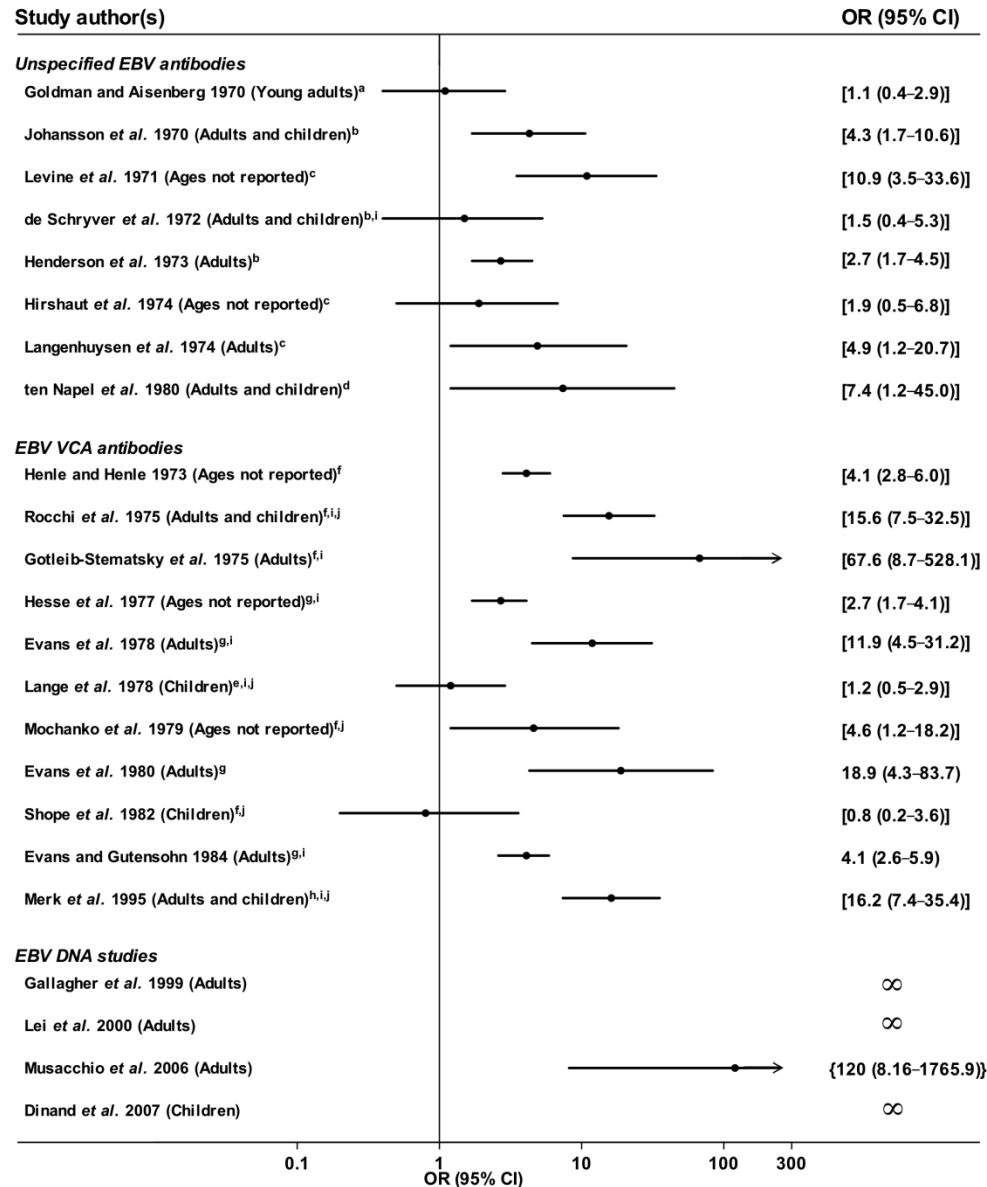
EBV protein expression

NA



Hodgkin Lymphoma

Serological case-control studies of Hodgkin lymphoma and EBV





Sufficient level of evidence from human studies

Epidemiology

Studies with positive associations or dose-response

EBV DNA: 4/4 case-control; very high ORs

EBV antibodies: 17/19 case-control & 1 nested case-control; mostly statistically significant OR between 4 & 19

Infectious mononucleosis: 10/11 case-control and 7/7 cohorts; modest ORs/RRs

Human tissue

Clonality for EBV

% EBV-infected tumors

Monoclonal

20-50% North America and Europe;
65% Asia; 90-100% Africa and South America

EBV protein expression

LMP-1, -2A in 50% cases



Sufficient evidence from human studies

| | NK/T-cell leukemia/lymphoma (nasal type) | Immunosuppression-related non- Hodgkin lymphoma |
|--|--|--|
| Epidemiology Studies with positive association | Consistent evidence in case series studies; at least 16 case-series with more than 400 cases 2 case-comparison studies: EBV DNA found in plasma CD3+ or CD3- cells of cases but not in controls | 2/2 case-control studies; nonsignificant increase in OR |
| Human tissue | | |
| Clonality for EBV | Monoclonal | Monoclonal |
| % EBV-infected tumors | 100% | 100% (primary CNS NHL, HIV+) 50% (diffuse large-cell and immunoblastic NHL, HIV+) >50% post transplant lymphoproliferative disease (PTLD) |
| EBV protein expression | EBNA-1, LMP-1, -2A | LMP-1, -2A, -2B, EBNA _s |
| Other | EBV found in majority of CD56+ tumors | Treatment with cytotoxic T-cells sensitized to EBV protect against or reduce viral load and tumor size in PTLD |

EBNA = Epstein-Barr nuclear antigen; LMP = latent membrane protein; NHL = non-Hodgkin lymphoma; CNS = central nervous system; HIV = human immunodeficiency virus.



Epithelial cancers

- Nasopharyngeal carcinoma
- Gastric cancer
- Lymphoepithelial carcinoma/salivary gland



Direct evidence

- EBV has been shown to transform lymphoblastoid cells in culture and can transform epithelial cells when co-cultured with transformed lymphoblastoid cells
- EBV infected B cells have been shown to cause B-cell lymphomas in immunodeficient (SCID) mice
- EBV proteins, EBNA-1, -2, -3A, -3C, LMP-1 are all necessary for immortalization of B-lymphocytes
 - EBNA-1 allows for increased survival and genomic instability; LMP-1 enables replicative immortality via NFkappaB pathway



EBV Mechanisms Epithelial Cancers

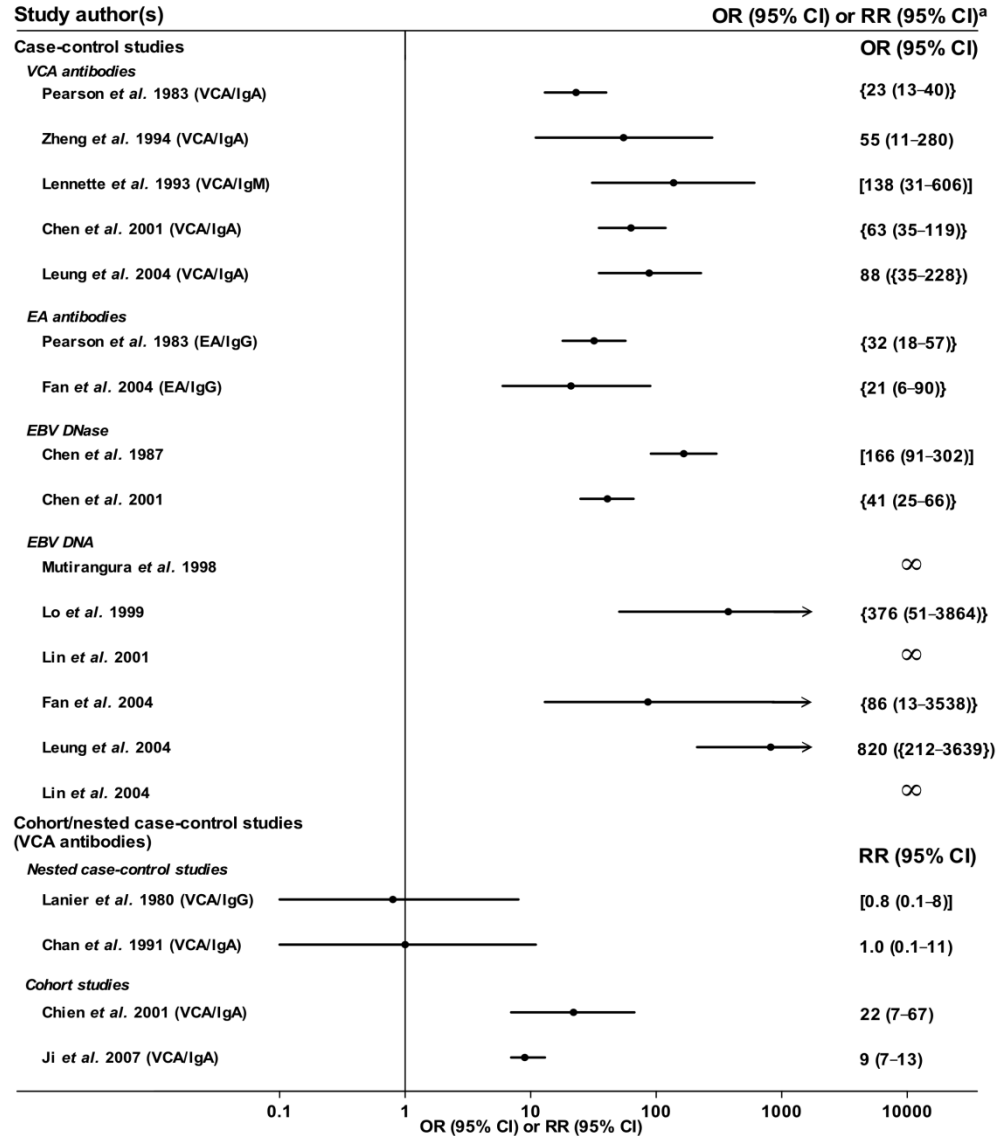
Latency II proteins expressed in epithelial cancers

- EBNA-1, LMP-1, -2A, EBERs
- Nasopharyngeal cancer
- Lymphoepithelial cancer of salivary glands
- Gastric cancer
 - Latency I and II patterns found in gastric cancers with approximately 50% expressing LMP-2A which enhances proliferation and survival factors



Nasopharyngeal Carcinoma

Serological case-control studies of nasopharyngeal carc. and EBV





Nasopharyngeal Carcinoma

Sufficient evidence from human studies

| | |
|---|---|
| Epidemiology Studies reporting positive associations | EBV antibody: 11/11 case-control and 2 cohort studies; high to very high statistically significant RRs; no association in 2 small nested case-control studies. EBV DNA: 6/6 case-control studies; very high RR |
|---|---|

| | |
|-----------------------------------|------------------------------------|
| Human tissue Clonality for EBV | Monoclonal in precancer and cancer |
| % EBV-infected tumors | 98% in nonkeratinizing tumors |
| EBV protein expression | EBNA-1, LMP-1,-2A |

EBNA = Epstein-Barr nuclear antigen; LMP = Latent membrane protein; RR = relative risk.



Sufficient level of evidence from human studies

Epidemiology

Studies with positive associations

Case-series

3/3 case-control studies (77 EBV cases/184 gastric); statistically significant high ORs

2/3 nested case-control studies; statistically significant modest ORs

Human tissue

Clonality for EBV

Monoclonal

% EBV-infected tumors

8 to 11%

EBV protein expression

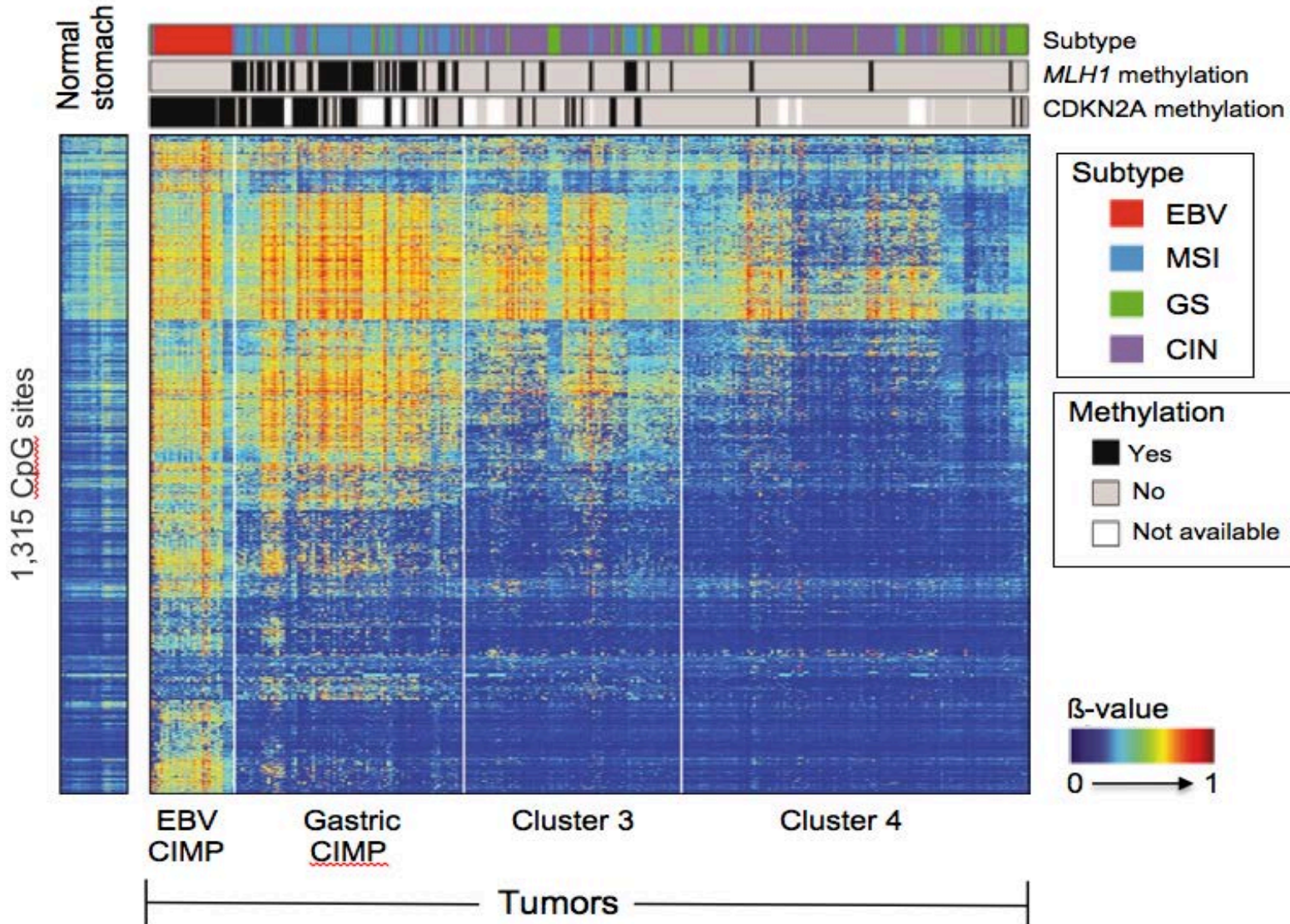
EBNA-1, LMP-1, -2A

Other

Unique molecular profile



Methylation Patterns in Human Gastric Cancer





Altered Pathways with EBV-related Gastric Cancer

| Signaling pathway | Biological effect |
|--------------------------|---|
| Micro RNAs | Unknown |
| CDKN2A (p16) | Tumor suppressor, slows G1 to S transition |
| JAK2 | Cell growth and division; LMP-1 activates |
| PI3K/Akt | Cell growth and division, inhibits apoptosis, promotes genomic instability; LMP-2 activates |
| ERBB2 | Cell growth and division |
| ARID1A | Cell-cycle progression |
| BCOR | Transcription and chromatin regulation |
| CD274 (PD-L1) | Immunosuppression |
| PDCD1LG2 (PD-L2) | Immunosuppression |
| IL-12 | Immune stimulation in response to antigen |
| NF-kappaB | Resists apoptosis; cell proliferation; LMP-2A activates |



Lymphoepithelial Carcinoma/Salivary Gland

Limited level of evidence from human studies

Epidemiology

Positive association

Consistent evidence in case series (208/209 cases)

A case-case study found EBV DNA in salivary gland lymphoepithelial carcinoma tumors but not other types of salivary gland tumors

Human tissue

Clonality for EBV

Monoclonal (evidence from one study)

% EBV-infected tumors

100%

EBV protein expression

EBNA-1, LMP-1,-2A (few samples)

No additional supporting mechanistic data.



EBV Preliminary Level of Evidence Summary

- Cancer sites with sufficient evidence
 - Burkitt lymphoma (endemic)
 - Hodgkin lymphoma
 - Nasopharyngeal cancer
 - Immunosuppression-related non-Hodgkin lymphoma
 - Extranodal NK/T-cell lymphoma (nasal type)
 - Gastric cancer
- Cancer sites with limited evidence
 - Burkitt lymphoma (sporadic)
 - Lymphoepithelial cancer of the salivary gland
- Latent viral transcripts enable cell survival, increase genomic instability, increase cell proliferation.



Clarifications?



All sections: Comment on whether the information is clear and technically accurate and identify any information that should be added or deleted,

Properties, Detection and Human Exposure

- and whether adequate information is presented to document past and/or current human exposure.

Human Cancer Studies

- and provide any scientific criticisms of NTP's cancer assessment of the epidemiologic studies of exposure to the virus.

Mechanistic and Other Relevant Data

- and provide any scientific criticisms of the NTP's synthesis of these data assessing effects of the virus.



Level of Evidence Conclusion (Vote)

EBV is known to be a human carcinogen based on sufficient evidence in humans.

- Cancer sites with sufficient evidence
 - Burkitt lymphoma (endemic)
 - Hodgkin lymphoma
 - nasopharyngeal cancer
 - immunosuppression-related non-Hodgkin lymphoma
 - extranodal NK/T-cell lymphoma (nasal type)
 - gastric cancer
- Cancer sites with limited evidence
 - lymphoepithelial cancer of the salivary gland
 - Burkitt lymphoma (sporadic)



Preliminary Listing Recommendation (Vote)

EBV

Epstein-Barr virus (EBV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans.

This conclusion is based on evidence from epidemiological, clinical, and molecular studies, which show that EBV causes endemic Burkitt lymphoma, Hodgkin lymphoma, immune-suppression-related non-Hodgkin lymphoma, extranodal natural killer –T-cell lymphoma nasal type, nasopharyngeal carcinoma, and some forms of stomach cancer.

There is also limited evidence for an association with sporadic Burkitt lymphoma and lymphoepithelial cancer of the salivary gland.



- Contains NTP's preliminary recommendation of the listing status of the substance.
- Summarizes the scientific information that is key to reaching a recommendation.
- Provides information on properties, use, production and exposure.
- Provides information on existing federal regulations and guidelines.



Peer Reviewer Comments

- Provide any new comments (e.g., not previously provided on the same facts or issues in the cancer hazard evaluation section) on whether the information on properties and detection, human exposure, cancer studies in humans and mechanistic data is clear and technically accurate.
- Comment on whether the substance profile highlights the information on cancer studies in humans and mechanistic data that are considered key to reaching the listing recommendation.