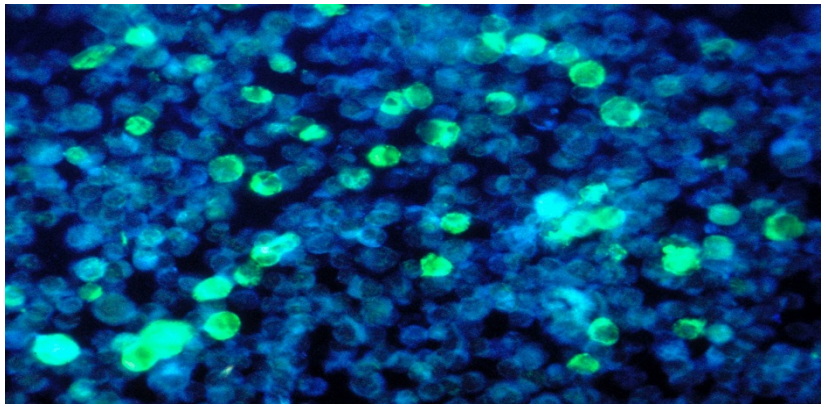
NTP
National Toxicology Program

Draft RoC Monograph
Epstein-Barr Virus



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Contract support to the ORoC

NTP Peer Review Meeting
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Outline

Properties

Detection

Transmission

Exposure

Mechanistic information

Human cancer studies

Preliminary level of evidence conclusions

EBV Properties

- 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)

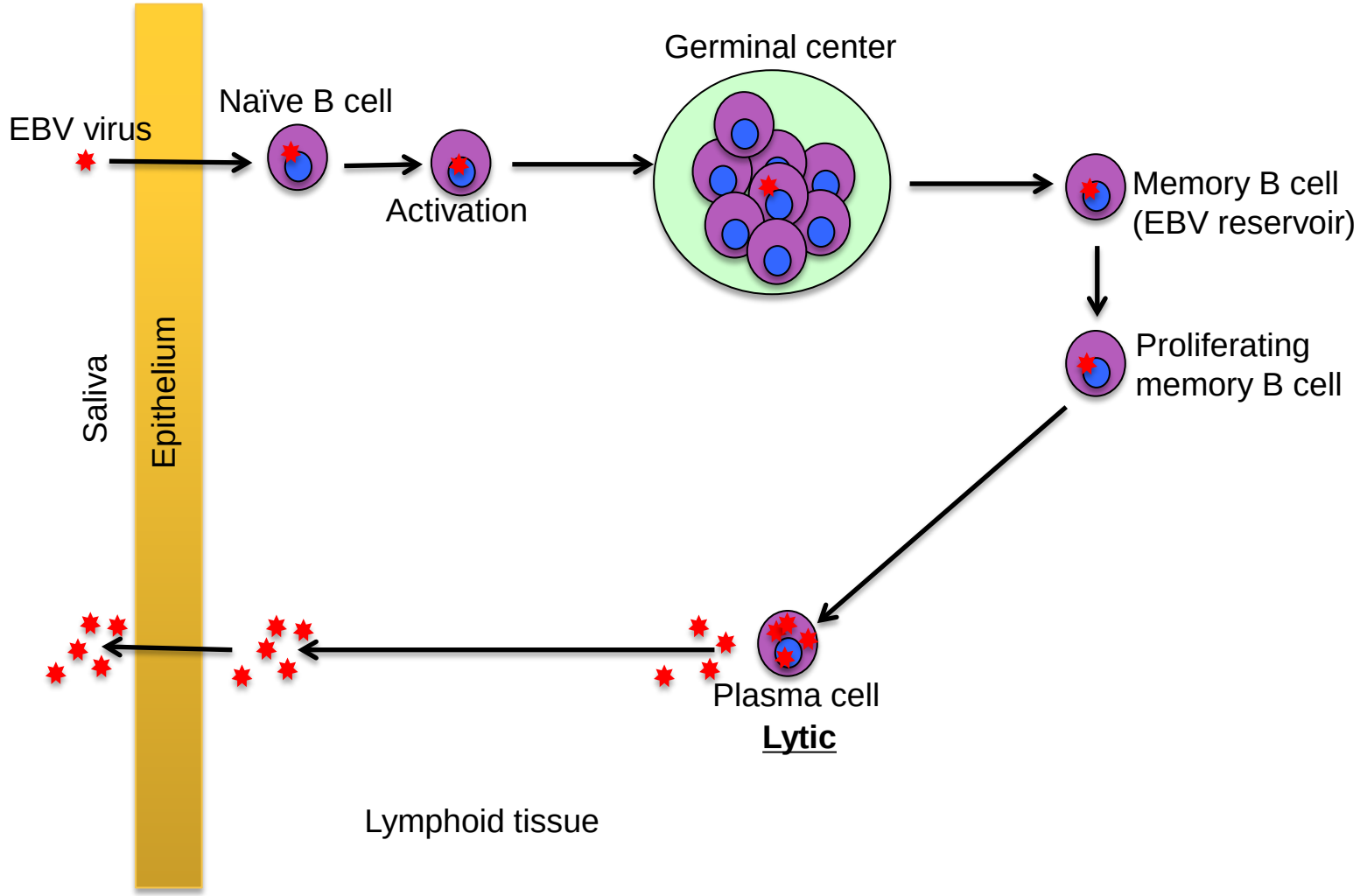
EBV Exposure

- 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



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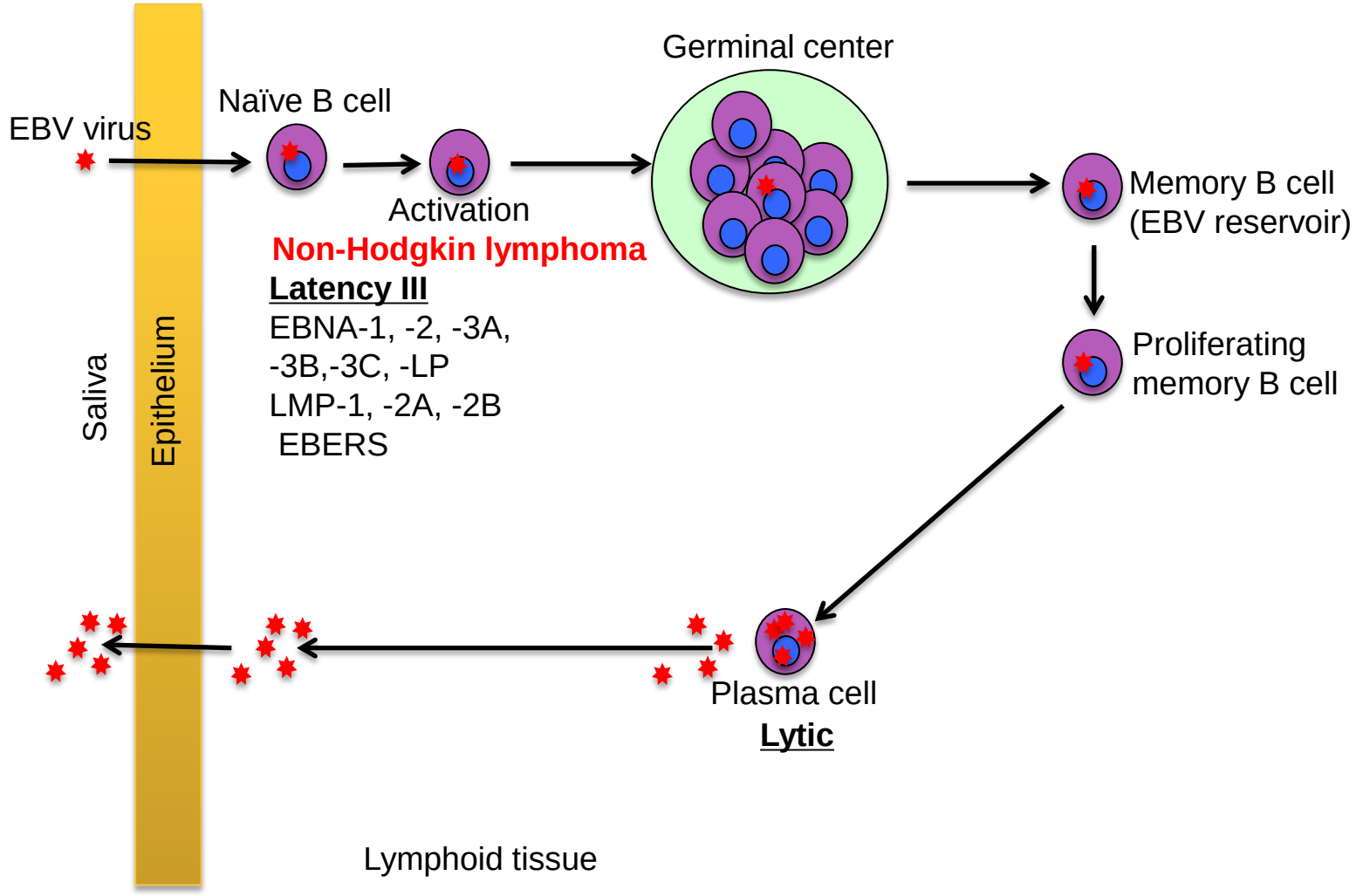

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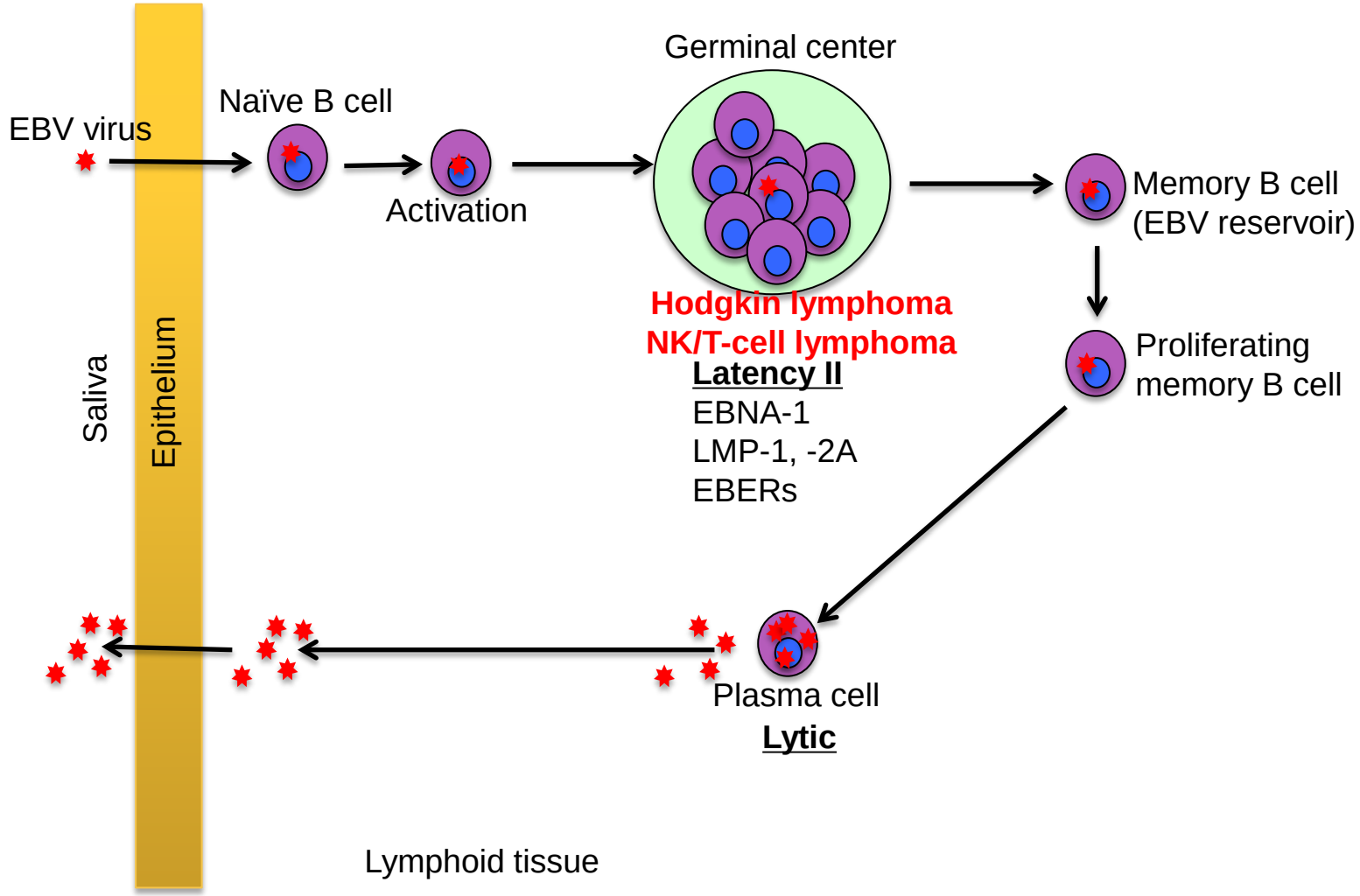

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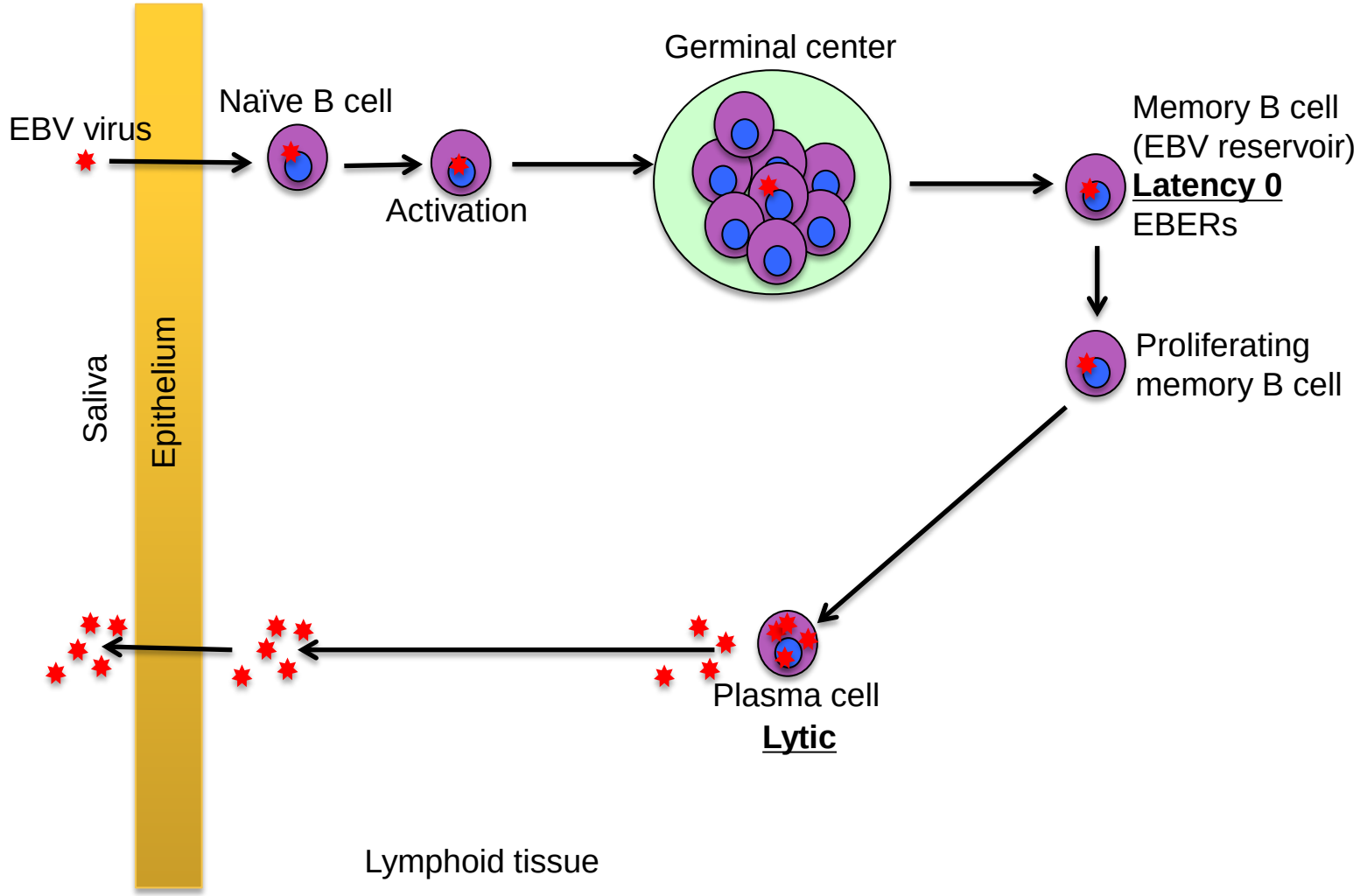

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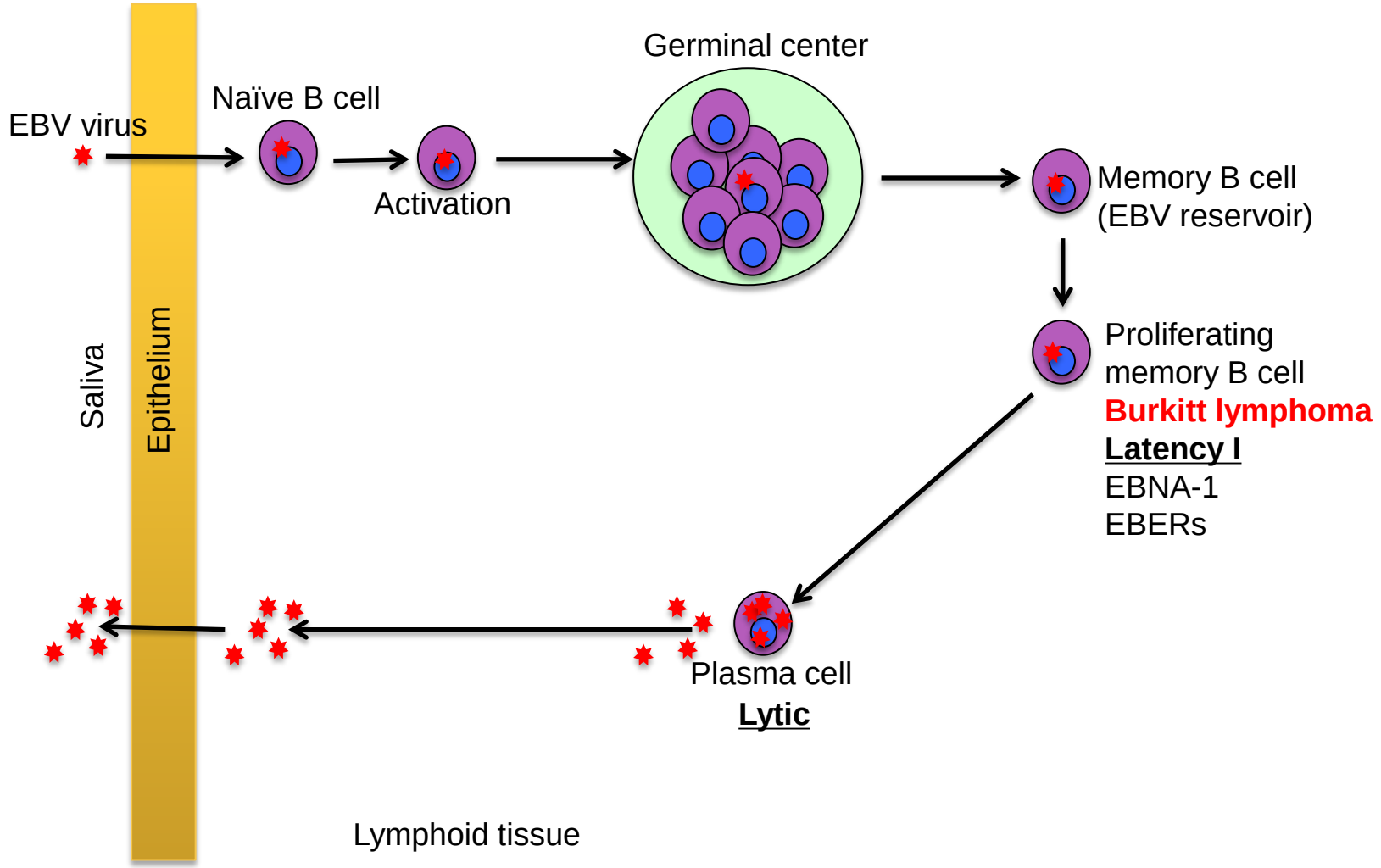


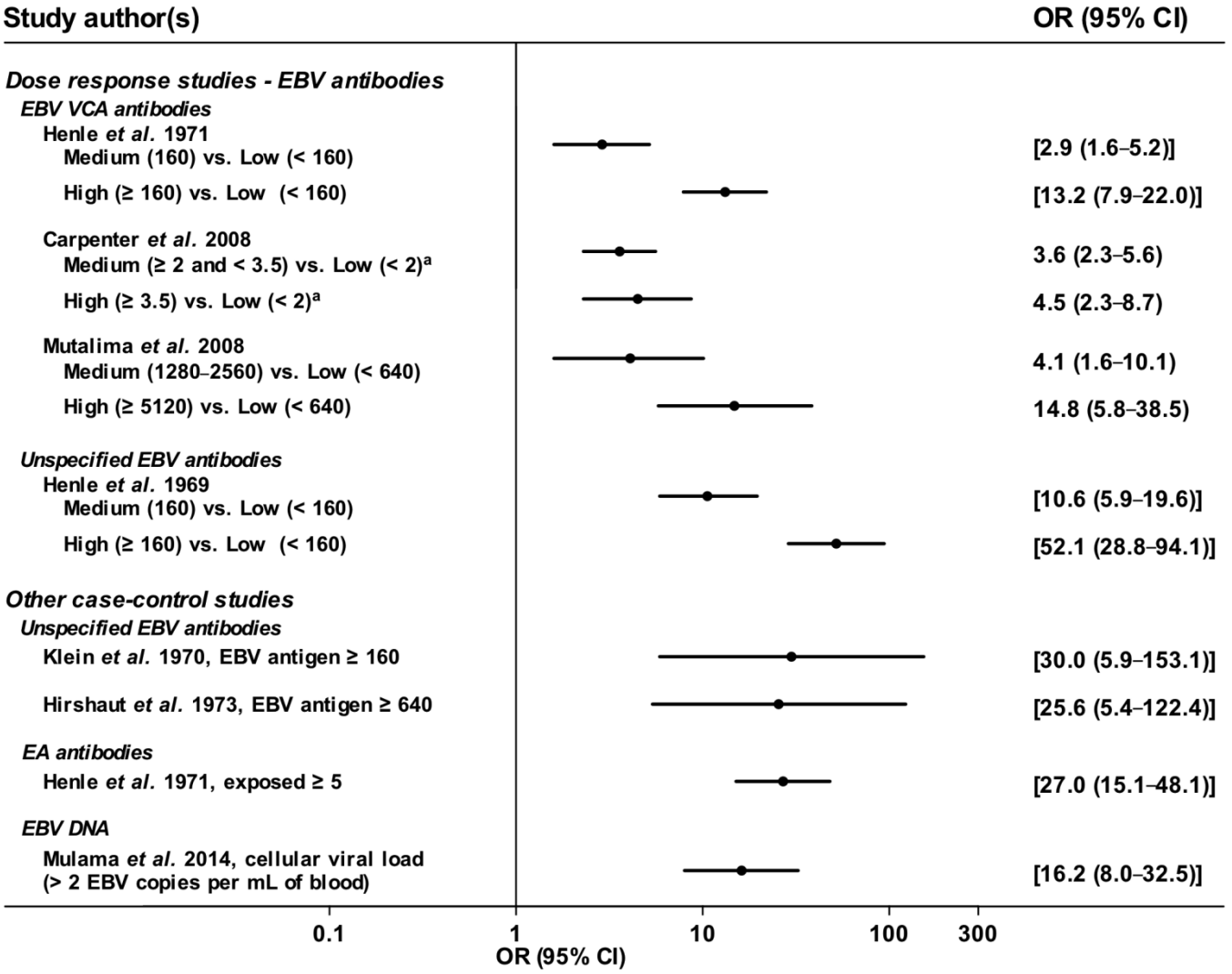
EBV and Human Cancer Studies

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

EBNA = Epstein-Barr nuclear antigen; OR = odds ratio; RR= relative risk.

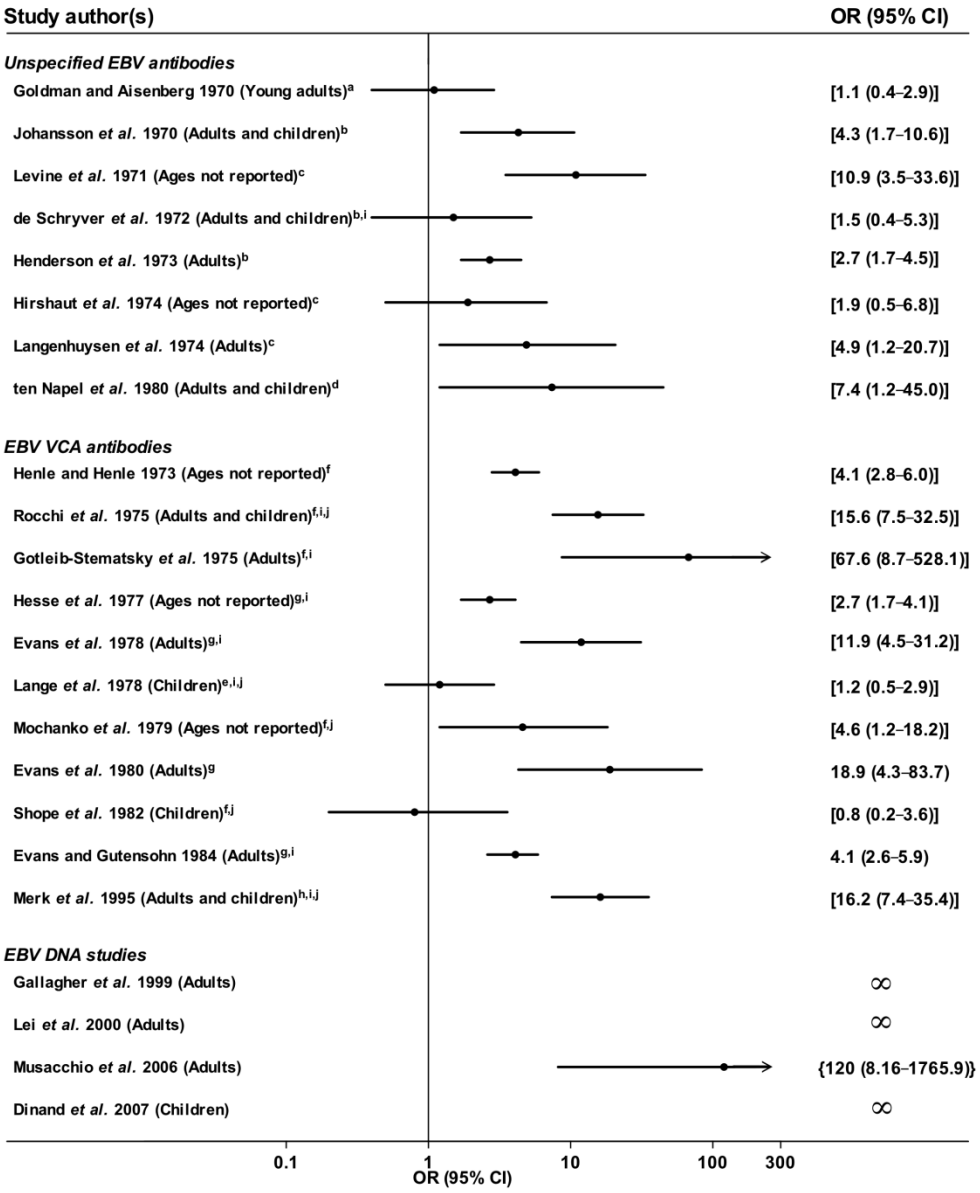
Burkitt Lymphoma (Sporadic)

Limited level of evidence for sporadic Burkitt lymphoma

Epidemiology	EBV antibodies: 4/5 case-control studies (113 cases).
Studies with positive associations or dose-response	Most not significant; moderate ORs
Human tissue	
Clonality	NA
% EBV-infected tumors	20%
EBV protein expression	NA

NA = not available; OR = odds ratio.

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	Monoclonal
% EBV-infected tumors	20-50% North America and Europe; 65% Asia; 90-100% Africa and South America
EBV protein expression	LMP-1, -2A in 50% cases

LMP- = latent membrane protein; OR = odds ratio; RR = relative risk.

Other Lymphomas

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, EBNAs
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.

EBV and Human Cancer Studies

Epithelial cancers

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

EBV Mechanisms in Epithelial Cancers

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

SCID = severe combined immuno-deficient.

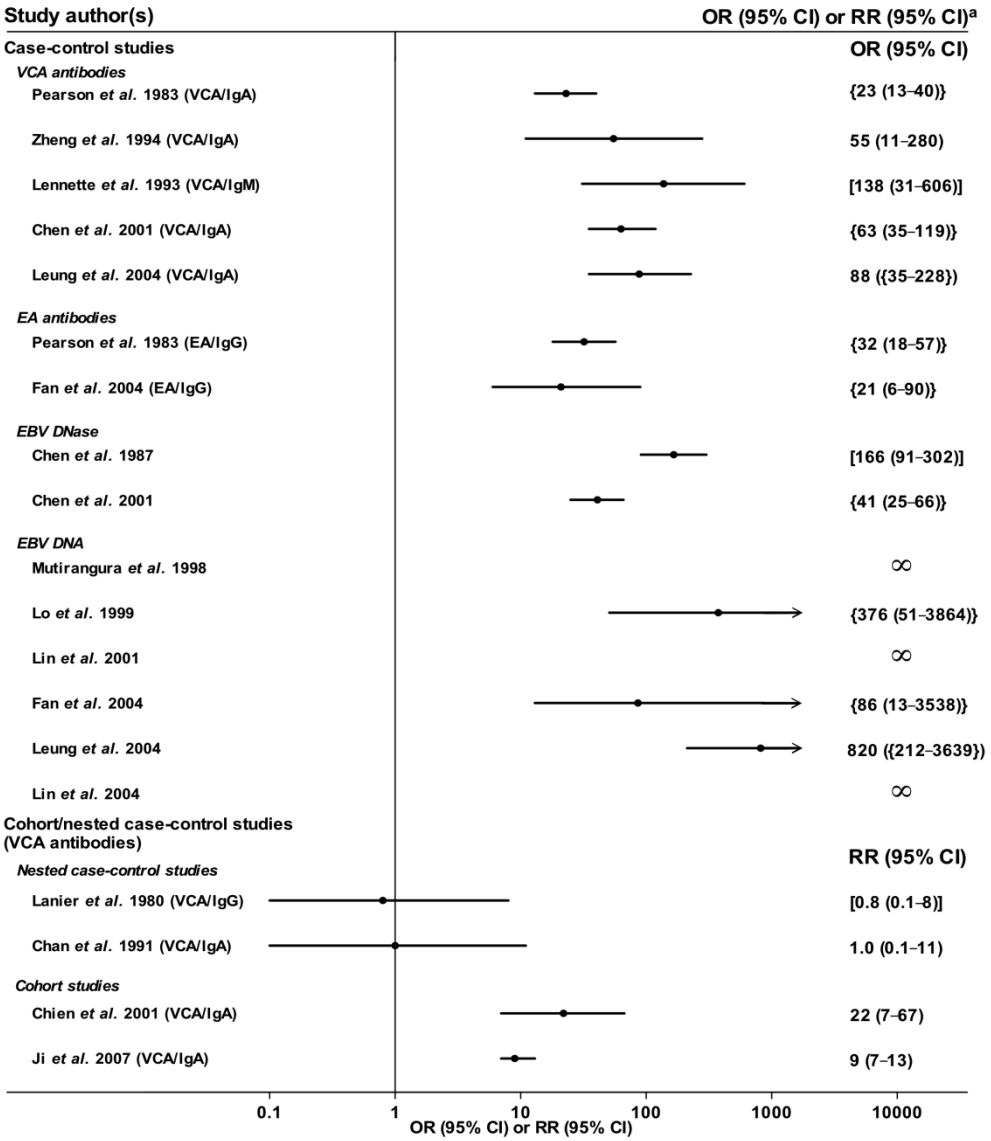
EBV Mechanisms Epithelial Cancers

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

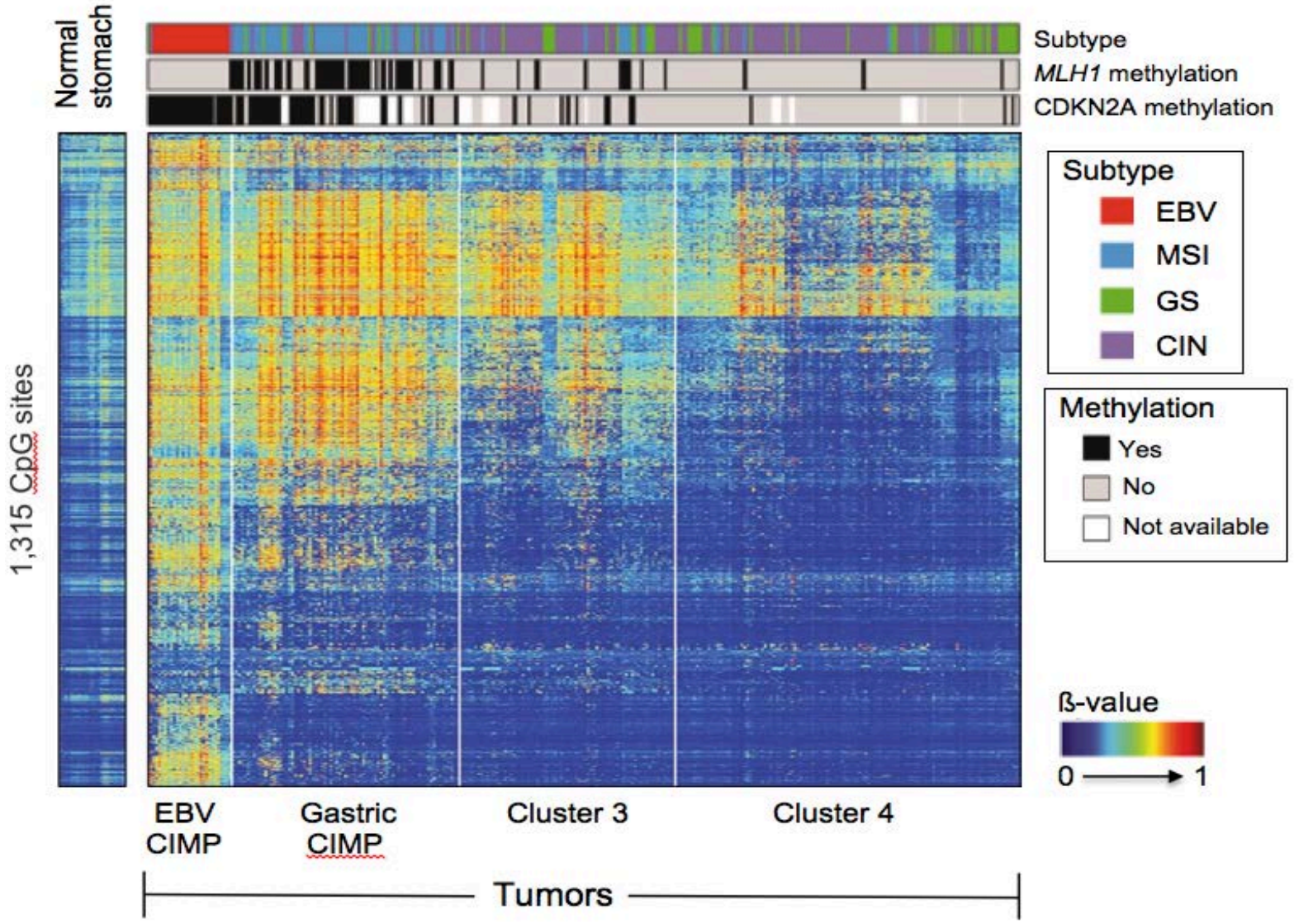
EBNA = Epstein-Barr nuclear antigen; LMP = Latent membrane protein; RR = relative risk.

Sufficient level of evidence from human studies

Epidemiology	Case-series
Studies with positive associations	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

OR = odds ratio; EBNA = Epstein-Barr nuclear antigen; LMP = Latent membrane protein

Methylation Patterns in Human Gastric Cancer



Modified from: The Cancer Genome Atlas Research Network (2014) *Nature* 513:202-209.

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	Consistent evidence in case series (208/209 cases)
Positive association	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.

EBNA = Epstein-Barr nuclear antigen; LMP = Latent membrane protein

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?

Peer Reviewer Comments

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.

Draft Substance Profile

- 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 informtion 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.