**Mission:** To provide scientific leadership and laboratory infrastructure to study immune responses to Human Papillomavirus (HPV) vaccines and other cancer preventive strategies in the context of clinical and pre-clinical studies.
Vaccine, Immunity and Cancer Program: HPV Serology Efforts

- Investigate immune responses to vaccines, infections, and cancer
- Develop and validate new methods for laboratory markers of protection
- Monitor immunity in clinical trials and pre-clinical studies
- Provide evidence to inform new trials and create tools to enable decision-making and public health changes

Support for NCI vaccine trials and epidemiological studies
- NCI Costa Rica Vaccine Trial

Collaboration with the extramural HPV vaccine community
- cCRADAs
  - Moffitt Cancer Center
  - University of London

HPV serology standardization initiative (reference lab)
- Partners: BMGF, CDC, PHE, NIBSC, KI, and WHO

Frederick National Laboratory for Cancer Research
Human Papillomavirus (HPV)

- Non-enveloped double-stranded DNA virus
- >200 types identified
- ~40 types sexually transmitted
  - 13 high-risk (oncogenic) types
    - HPV-16 and HPV-18 types account for 70% of cervical cancer cases
  - Low-risk (non-oncogenic) types
    - HPV-6 and HPV-11 associated with >90% of anogenital warts

Most common sexually transmitted infection
Worldwide Incidence and Distribution of Cancers Attributable to HPV

Cervical cancer accounts for ~ 83% of HPV attributable cancers globally.

Estimated cancer cases attributable to HPV ~ 5%

1 woman dies of cervical cancer every 2 minutes

Adapted from de Martel et al., Int J Cancer 2017;141(4): 664, GLOBOCAN
USA: HPV-Associated Cancers Affect Both Sexes

Cancers attributable to HPV infection

- **MEN**
  - Cervical
  - Penile
  - Oropharyngeal

- **WOMEN**
  - Cervical
  - Vulvar/Vaginal
  - Anal
  - Non-cervical cancers

**Annual number of cases**

- Total number of HPV-positive cancers = ~34,800, ~60% women; ~40% men
- HPV-16/18: Accounts for ~70% of cervical cancers, ~90% of non-cervical cancers

Adapted from Senkomago et al., MMWR, Vol 68, #33, August 23, 2019
HPV and HPV-Associated Cancers Are Preventable: Three Licensed HPV Prophylactic Vaccines

<table>
<thead>
<tr>
<th>HPV L1 VLP types</th>
<th>Quadrivalent (Gardasil®) Merck</th>
<th>Bivalent (Cervarix®) GSK</th>
<th>Nonavalent (Gardasil®9) Merck</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-6/11/16/18</td>
<td>HPV-16/18</td>
<td>HPV-6/11/16/18/31/33/45/52/58</td>
<td></td>
</tr>
<tr>
<td>FDA Approval</td>
<td>2006</td>
<td>2009</td>
<td>2014</td>
</tr>
<tr>
<td>Sex/age groups</td>
<td>Females and males 9-26 yrs</td>
<td>Females 9-25 yrs</td>
<td>Females and males 9-26 yrs</td>
</tr>
<tr>
<td>Licensed*</td>
<td>3 doses</td>
<td>3 doses</td>
<td>3 doses</td>
</tr>
<tr>
<td>Schedule</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected Coverage</td>
<td>70% of Cervical Cancers</td>
<td>70% of Cervical Cancers</td>
<td>90% of Cervical Cancers</td>
</tr>
<tr>
<td></td>
<td>90% Genital Warts</td>
<td></td>
<td>90% Genital Warts</td>
</tr>
</tbody>
</table>

* New recommendation approved by FDA (Oct 2016): 2 doses (0 and 6 month), 9-14 yrs

# New approval by FDA (Oct 2018): 27-45 yrs for Gardasil®9
High Efficacy of HPV Prophylactic Vaccines Against New Cervical Precancer and Genital Warts in Randomized Trials

In women with no genital HPV infection detected at the start of each trial

<table>
<thead>
<tr>
<th>End Point</th>
<th>Sex</th>
<th>Age</th>
<th>Vaccine</th>
<th>Targeted HPV Types</th>
<th>Efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precancer</td>
<td>Female</td>
<td>15-26</td>
<td>Quadrivalent/ Gardasil</td>
<td>HPV-6/11/16/18</td>
<td>100% (85.5-100)</td>
</tr>
<tr>
<td>Genital Warts</td>
<td>Female</td>
<td>15-26</td>
<td>Quadrivalent/ Gardasil</td>
<td>HPV-6/11/16/18</td>
<td>96.4% (91.4-98.4)</td>
</tr>
<tr>
<td>Precancer</td>
<td>Female</td>
<td>15-25</td>
<td>Bivalent/ Cervarix</td>
<td>HPV-16/18</td>
<td>100% (90.5-100)</td>
</tr>
<tr>
<td>Precancer</td>
<td>Female</td>
<td>16-26</td>
<td>Nonavalent/ Gardasil 9</td>
<td>HPV-6/11/16/18/ 31/33/45/52/58</td>
<td>97.1% (83.5-99.9)</td>
</tr>
</tbody>
</table>

Precancer = Cervical Intraepithelial Neoplasia Grade 3

Lehtinen et al., Lancet Oncol 2011; Munoz et al., JNCI 2010; Huh et al., Lancet 2017
HPV Vaccine Immunogenicity: A Critical Parameter in HPV Vaccine Trials

- **HPV serology** is a critical tool for assessment of vaccine immunogenicity, which is a **key parameter** along with clinical efficacy (protection against infection/or disease) in vaccine trials.

- **HPV neutralizing antibodies** are the main **mechanism of protection** against infection induced by prophylactic vaccines.

- Vaccination in animal papillomavirus models have implicated antibodies as immune effectors.
  
  - **Protection against infection** and subsequent lesion development can be **passively transferred** by serum antibodies.
HPV Immunology Laboratory Support to HPV Vaccine Trials: Immunogenicity Studies

- Phase III Trial of the Bivalent HPV Vaccine in Healthy Young Adult Women in Costa Rica (NCI Costa Rica Vaccine Trial) (PI: Dr. Allan Hildesheim, NCI)
  - Women, ages 18-25 years (n=7,466) (NCT00128661, NCT00867464)

- Safety and Immunogenicity Trial in Adult Volunteers of an HPV-16 L1 VLP Vaccine (PI: Dr. Clayton Harro, The Johns Hopkins University)
  - Women, ages 18-29 years (n=72)

- Phase II Trial of the Quadrivalent HPV Vaccine in Mid-Adult Males (MAM) (PI: Dr. Anna Giuliano, Moffitt Cancer Center)
  - Men, ages 27-45 years (n=150) (NCT01432574)

- Developed and optimized assays for measuring markers of protection against infection, both in blood and at sites of infection
- Monitored duration of antibody responses to vaccination
  - Neutralizing antibodies are the main mediators of protection
- Demonstrated long-term antibody responses even in single dose vaccine recipients – suggesting a single dose may work
Validated Assays for Measurement of Antibodies (In Serum and at Mucosal Sites)

HPV Serology Assays

- **Quantity:**
  - HPV IgG ELISA
    - HPV-16/18 L1 VLP
  - Luminex-based multiplex assay

- **Quality/Function:**
  - Pseudovirion neutralization assay
    - HPV-16/18/31/45/52/58
  - ELISA antibody avidity assay
  - Memory B cell ELISPOT

*All assays use in-house produced HPV VLPs, standards, and controls*
ELISA cutoff: HPV-16 = 8 EU/ml
HPV-18 = 7 EU/ml

Adapted from Safaeian et al., Cancer Prevention Research 2013; 6(11): 1242
Strong Correlations between ELISA and Neutralization Assays

LaMontagne et al., Vaccine 2014; 32 (47):6303
Antibody Levels at the Cervix Correlate with Systemic Levels

NCI Costa Rica Vaccine Trial (CVT)

HPV-16

N= 42
Spearman= 0.73
p< .0001

HPV-18

N= 42
Spearman= 0.75
p< .0001

Kemp et al., Vaccine 2008; 26: 3608
Antibody Levels in Saliva Correlate with Systemic Levels

HPV-16 antibody 1 month post-dose 3 of quadrivalent HPV vaccine
(Cohort: Mid-Adult Male Vaccine Trial, Moffitt Cancer Center)

\[ \rho = 0.90, \ p < 0.001 \]
\[ \rho = 0.92, \ p < 0.001 \]

Pinto et al., J Infect Dis. 2016, 214(8):1276
One Dose of the Bivalent HPV Vaccine Is Effective in Preventing HPV Infection Compared With Multiple Doses (48 Months After Vaccination)

**Cohort:** NCI Costa Rica Vaccine Trial, HPV-16/18 DNA negative at 1\textsuperscript{st} vaccination

**Endpoint:** Incident HPV-16/18 infections that persist for >6 months

<table>
<thead>
<tr>
<th># of Doses</th>
<th>Arm</th>
<th># of Women</th>
<th># of Persistent HPV-16/18 Infections</th>
<th>HPV-16/18 VE (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Control</td>
<td>3010</td>
<td>133</td>
<td>81% (71% to 88%)</td>
</tr>
<tr>
<td></td>
<td>HPV</td>
<td>2957</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>380</td>
<td>17</td>
<td>84% (50% to 96%)</td>
</tr>
<tr>
<td></td>
<td>HPV</td>
<td>422</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>188</td>
<td>10</td>
<td>100% (67% to 100%)</td>
</tr>
<tr>
<td></td>
<td>HPV</td>
<td>196</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

VE = Vaccine Efficacy

*p trend = 0.2*
The Bivalent HPV Vaccine Induces Durable Antibody Responses in One Dose Recipients

Safaeian et al., Cancer Prevention Research 2013; 6(11): 1242
Stable HPV-16 Serum Antibodies a Decade After One Dose of the Bivalent HPV Vaccine (NCI Costa Rica Vaccine Trial)

100% of 1 dose recipients remain seropositive at 11 years

One dose schedules may help reduce the current programmatic and economic challenges

Kreimer et al., Submitted; Safaeian et al., JNCI 2018; 110 (2): 205
HPV Vaccination Status: Current Global HPV Vaccine Demand/Supply Imbalance

- Only ~50% of countries with national immunization programs
- Introductions are lowest in low- and middle-income countries (LMIC)
- Very low global vaccine uptake
- WHO call for action towards global cervical cancer elimination will increase demand over the next 10 years
- Imbalance projected to last 3-5 years

Introduction Status (% of countries)

- Only ~50% of countries with national immunization programs
- Introductions are lowest in low- and middle-income countries (LMIC)
- Very low global vaccine uptake
- WHO call for action towards global cervical cancer elimination will increase demand over the next 10 years
- Imbalance projected to last 3-5 years
New One Dose Trials to Evaluate Immunogenicity and Non-Inferiority at FNL to Accelerate Policy Recommendations (2020-2025)

Scientific evaluation of one or two doses of the bivalent or nonavalent prophylactic HPV vaccines (ESCUDDO): (Dr. Aimee Kreimer, NCI), 20,000 girls, ages 12-16, NCT03180034

Kenya single dose HPV vaccine efficacy (KEN SHE): (Dr. Ruanne Barnabas, University of Washington), 2,250 women, ages 15-20, NCT03675256

A dose reduction immunobridging and safety study of two HPV vaccines in Tanzanian girls (DoRIS): (Dr. Deborah Watson-Jones, University of London), 900 girls, ages 9-14, NCT02834637

Non-inferiority trial comparing immunogenicity from 1 dose of bivalent HPV vaccine in girls to 3 doses of quadrivalent vaccine in women (PRIMAVERA): (Dr. Aimee Kreimer, NCI), 620 girls, ages 9-14 and 620 women, ages 18-25, NCT03728881

Phase IIA trial of a nonavalent prophylactic HPV vaccine to assess immunogenicity of a prime and deferred-booster dosing schedule among 9-11 year-old girls and boys: (Drs. Yi Zeng, Anna-Barbara Moscicki and Vikrant Sahasrabuddhe), 200 boys and girls, ages 9-11, NCT02568566
## Increasing Number of Prophylactic HPV Vaccines in Development - China

<table>
<thead>
<tr>
<th>Company</th>
<th>Vaccine</th>
<th>Expression system</th>
<th>IND</th>
<th>P I</th>
<th>P II</th>
<th>P III</th>
<th>BLA</th>
<th>MKT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  GSK</td>
<td>HPV-2 (16, 18)</td>
<td>Insect cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2  Merck</td>
<td>HPV-4 (6,11,16,18)</td>
<td>Yeast (S. cerevisiae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3  Merck</td>
<td>HPV-9 (6,11,16,18,31,33,45,52,58)</td>
<td>Yeast (S. cerevisiae)</td>
<td></td>
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<tr>
<td>4  Innovax</td>
<td>HPV-2 (16,18)</td>
<td>E.coli</td>
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<tr>
<td>5  Zerun</td>
<td>HPV-2 (16,18)</td>
<td>Yeast (P. pastoris)</td>
<td></td>
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</tr>
<tr>
<td>6  CNBG/CDIBP</td>
<td>HPV-4 (6,11,16,18)</td>
<td>Yeast (H. polymorpha)</td>
<td></td>
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<tr>
<td>7  Innovax</td>
<td>HPV-2 (6,11)</td>
<td>E.coli</td>
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</tr>
<tr>
<td>8  Kangleweishi</td>
<td>HPV-3 (16,18,58)</td>
<td>E.coli</td>
<td></td>
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</tr>
<tr>
<td>9  Bovax</td>
<td>HPV-4 (6,11,16,18)</td>
<td>Yeast (H. polymorpha)</td>
<td></td>
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</tr>
<tr>
<td>10 Bovax</td>
<td>HPV-9 (6,11,16,18,31,33,45,52,58)</td>
<td>Yeast (H. polymorpha)</td>
<td></td>
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<tr>
<td>11 Zerun</td>
<td>HPV-9 (6,11,16,18,31,33,45,52,58)</td>
<td>Yeast (P. pastoris)</td>
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<tr>
<td>12 Innovax</td>
<td>HPV-9 (6,11,16,18,31,33,45,52,58)</td>
<td>E.coli</td>
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</tr>
<tr>
<td>13 CNBG/SIBP</td>
<td>HPV-4 (6,18,52,58)</td>
<td>Yeast (P. pastoris)</td>
<td></td>
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</tr>
<tr>
<td>14 Kangleweishi</td>
<td>HPV-9 (6,11,16,18,31,33,45,52,58)</td>
<td>E.coli</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>15 Jiangsu Ruike</td>
<td>HPV-9 (6,11,16,18,31,33,45,52,58)</td>
<td>Yeast (H. polymorpha)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 CNBG/CDIBP</td>
<td>HPV-11 (6,11,16,18,31,33,45,52,58,59,62)</td>
<td>Yeast (H. polymorpha)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Nuoning</td>
<td>HPV-14 (6,11,16,18,31,33,35,39,45,51,52,56,58,59)</td>
<td>Insect cell</td>
<td></td>
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</tbody>
</table>

Data source: NMPA data; clinicaltrial.gov. updated in April, 2019

Prepared by Peter Dull, BMGF
Transition from Clinical to Serology Endpoints in Vaccine Trials: Increased Demand for Standardized Serology Testing

- Increasing number of clinical trials are proposing to use serology as endpoints
- No commercially available validated assays
- Lack of uniform, standardized assays, procedures, and reagents accessible to the vaccine trial community
- Vaccine trials have used different serologic assays, different VLP production methods and different reporting units
- **Difficulty in comparing results across studies**
The HPV Serology Standardization Initiative - 2017 (Sponsored by NCI and The Bill & Melinda Gates Foundation)

Mission:
• To work in partnership with the international HPV serology community to promote further standardization, harmonization and proficiency of HPV serology assays to assess vaccine immunogenicity in vaccine trials through:
  • development of qualified assay standards, critical reagents (HPV Virus-Like Particles), multiplex assays and guidelines available to the scientific community

Impact:
• Enable comparisons of data between different vaccines and studies
• Accelerate implementation of new vaccines and new vaccine recommendations

Partners:
Frederick National Laboratory: Ligia Pinto, Troy Kemp
NCI: Doug Lowy, John Schiller, Sean Hanlon
The Bill & Melinda Gates Foundation: Peter Dull
CDC: Elizabeth Unger
Karolinska Institute: Joakim Dillner
Public Health England: Simon Beddows
Biostat Consulting, LLC: Brian Plikaytis

https://frederick.cancer.gov/science/hpvserologylab/overview
HPV Serology Standardization: Progress to Date

- Generated **qualified secondary standards** and bank of specimens for use as assay proficiency panels
- Completed production of **qualified reference Virus-Like Particles** for the 9 HPV types included in the nonavalent HPV vaccine
- Developed and validated **Multiplex Serology Assays** for vaccine trial use
- **Promote use of standards** (meetings, publications, data, and protocol sharing)
  - FNL developed a **website** with all Standard Operating Procedures created in-house
  - Host **meetings** at the International Papillomavirus Conferences
  - **20 Material Transfer Agreements (MTA)**
HPV Serology Standardization: Collaboration Across the Globe
(20 Material Transfer Agreements)
Main Achievements Working Together With NCI and Extramural Collaborators

- Contributed to more than **93 peer-reviewed publications** (52 in the area of HPV)
- Established four **Contractor Cooperative Research and Development Agreements (cCRADAs)** to study immune responses to vaccination in females and males
- Provided laboratory evidence that stimulated the ongoing large **NCI single dose efficacy trial of the HPV vaccine in Costa Rica** (20,000 participants) and other single dose HPV vaccine trials
- Provided preliminary evidence to support HPV vaccine **efficacy trials in adult men living with HIV**
- Established an **international HPV serology standardization initiative** to reliably measure immune responses to vaccination and enable comparisons of data between different studies
Impact of Our Work: How Are We Contributing to the Elimination of Cervical Cancer and Other HPV-Associated Cancers?

- Investigate immune responses to vaccines, infections, and cancer
- Develop and validate new methods for laboratory markers of protection
- Monitor immunity in clinical trials and pre-clinical studies
- Provide evidence to inform new trials and create tools to enable public health changes

- Understanding how HPV vaccines work and mechanisms associated with protection
- Providing immuno-epidemiological evidence to move forward with new trials for novel vaccine recommendations
- Leading an international HPV serology standardization initiative to enable use of laboratory markers for demonstration of vaccine effectiveness
- Supporting development and evaluation of new candidate vaccines
HPV Serology Standardization: Future Plans

- Establishment of a **Center of Excellence for HPV Serology**
- Implement **high throughput HPV antibody testing core unit, with Good Clinical Laboratory Practice (GCLP) capability** to support NCI trials and extramural vaccine trial community
- Build an **HPV Vaccine Trial Network** of laboratories involved in serological testing of HPV vaccines
  - Develop training capability
  - Develop an Assay Proficiency Panel Program
- Continue **working with NIBSC and WHO** for development of WHO International Standards as well as with all the other stakeholders
Acknowledgments

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HPV Immunology Laboratory
- David Pan
- Marcus Williams

HPV Serology Laboratory
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- Angelina Richards

Moffitt Cancer Center
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The Bill & Melinda Gates Foundation
- Peter Dull

Division of Cancer Epidemiology and Genetics, NCI

- Allan Hildesheim
- Mahboobeh Safaeian
- Aimee Kreimer

Laboratory of Cellular Oncology, NCI
- Douglas Lowy
- John Schiller

Center for Strategic Scientific Initiatives, NCI
- Sean Hanlon

Division of Cancer Prevention, NCI
- Vikrant Sahasrabuddhe